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OF
THE JOHNS HOPKINS HOSPITAL

(THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL)

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THE ELECTROMYOGRAM IN MYASTHENIA GRAVIS

A. M. HARVEY[1] AND R. L. MASLAND

From the Institute of Neurology and the Johnson Foundation, University of Pennsylvania, Philadelphia

Submitted for publication April 30, 1941

Since the discovery by Walker (1) that the muscle weakness of myasthenia gravis may be promptly relieved by eserine, there has been much speculation concerning the nature of the fundamental disturbance in this condition. At present, however, we have little knowledge as to the location of the defect or as to its nature. The known action of eserine in altering the function of choline esterase in the tissues has suggested that its effect in myasthenia indicates a disturbance in the processes concerned with neuromuscular transmission. However, deductions made from a study of a single action of a drug are often unreliable, and the mere fact that eserine improves muscle power in myasthenia does not prove that the fundamental disturbance is at the neuromuscular junction. It is known that eserine is capable of stimulating motor nerve endings (2), and that it also has an action on spinal reflexes (3). To assume that its action as an anti-choline esterase is the effective one in myasthenia would appear unwarranted. Furthermore, choline esterase determinations in patients with myasthenia have been found to be within normal limits (4).

In 1933, Pritchard (5) studied the mechanical responses of the myasthenic muscle during stimulation of the motor nerve. No abnormality was found in the response evoked by a single nerve stimulus. With rates of stimulation around 40 per second, continued for from 1 to 5 seconds, the response was maintained in the normal fashion. When the frequency was increased to 80 per second, the contraction fell off rapidly, and was maintained at less than the original height. As the frequency of stimulation was increased still further, the decrease in the response became progressively more marked. A falling off in

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the contraction at these rates of stimulation was not seen in normal subjects. Pritchard suggests that this is an example of Wedensky inhibition, and that it affords our only direct evidence that the muscular weakness in myasthenia is probably due to a disturbance in transmission of excitation from motor nerve to voluntary muscle. These experiments are open to certain objections. There is no way of knowing that the nerve stimuli were maximal, and if they were submaximal, the responses would be subject to unpredictable variations due to changes in nerve threshold. Furthermore, the abnormalities in the responses obtained were not sufficiently characteristic to be used as a criterion for differentiating between changes in nerve activity, junctional transmission, or muscle function.

Several studies have been made in patients with myasthenia gravis of the muscle action potentials in response to nerve stimulation. In no instance, however, are the records reported suitable for quantitative comparison with the normal, for no effort has been made to insure maximal stimulation of the motor nerve. Herzog (6) stimulated the ulnar nerve and recorded from the hypothenar eminence. He found that, with a stimulation frequency of 64 per second, the action potentials of the successive responses tended to become smaller. This decrease in size tends to confirm the myographic findings of Pritchard. However, Harvey and Masland (7) have shown that, at this frequency, the action potentials of normal subjects may show an appreciable decline in size. Furthermore, the records reproduced in Herzog's report show a rapid change in amplitude which we have occasionally observed as a result of displacement of the stimulating electrodes.

Eichler and his co-workers (8) recording from the peroneus group and also from the muscles innervated by the median nerve, found that the action potential of the myasthenic muscle was of low voltage and increased duration, and resembled that seen in fatigue. They state that the conduction velocity of the nerve is slower than normal, and that the negativity of both phases of the potential is of increased duration. In their records, the muscle action potential is preceded by a large stimulus artefact which encroaches on the true response, making it difficult to interpret. Furthermore, no precautions were taken to insure maximal stimulation of the nerve.

Lindsley (9) has made myographic and electromyographic studies

of four patients suffering from myasthenia gravis. Using coaxial needle electrodes, recording the response of individual muscle fibres or of motor units, he found that although the rhythm of discharge of the motor units on voluntary contraction was normal, the size of the potential recorded from the unit was subject to wide variation. This variation was greater with fatigue of the muscle, and was abolished after administration of prostigmin. He believed that these studies indicated that in myasthenia gravis the motoneurone is functioning normally. The variation in size of the response is due to intermittent blocking of transmission of the nerve impulse across the neuromuscular junction. Lindsley also applied single maximal shocks to the ulnar nerve, and recorded the contraction of the fifth finger mechanically. The tension developed was increased by the administration of prostigmin. In contrast to the findings of Pritchard, even at a frequency of stimulation of 10 per second, prostigmin increased the height of the myographic curve.

Recently we have reported (7) a method for studying quantitatively the electrical responses of human muscle during stimulation of the motor nerve. Studies have been made of the characteristics of the muscle action potential of the normal subject, and changes in this potential during the administration of curarizing substances have been observed. The present report deals with the results obtained by the application of this method to the study of three patients suffering with myasthenia gravis.

METHOD AND MATERIAL

As previously described (7), electrodes are placed on the skin over the base of the proximal phalanx of the fifth finger and over the hypothenar eminence. Through these electrodes are recorded the muscle action potentials in response to maximal stimulation of the ulnar nerve. The position of the electrodes is such that they record the potential between the belly and tendon of the abductor digiti quinti muscle. Variations in the size of this potential are indicative of variations in the number of muscle fibers contracting. As long as the nerve stimulus is supra-maximal, and the duration of the potential does not increase, any decrease in the size of the total muscle action potential is an indication that some of the muscle fibers are failing to respond to an impulse in their motor nerve.

Three patients were studied, all presenting the typical picture of myasthenia gravis. In each, the administration of prostigmin was followed by dramatic improvement. As a further test, two of the patients were each given two doses of

quinine sulphate, 0.6 gram, and showed marked exacerbation of symptoms following this medication. Myasthenia gravis is the only condition known in which the symptoms are relieved by prostigmin and enhanced by the administration of small amounts of quinine (10).

RESULTS

Response to a single nerve volley

The action potential recorded from the muscle of a myasthenic individual following a single nerve stimulus is identical with that seen in normal subjects under similar circumstances. In the myasthenic, as in the normal, the usual diphasic response, with slight notching of the negative deflection is always seen. Measurements of the size and also the duration of the action potential are similar for the two conditions.

An abnormality was observed in the changes in the single response which appeared during a period of arterial occlusion in the limb. Arterial occlusion with a blood pressure cuff for periods up to 10 minutes in the normal subject produces little change in the action potential. When the circulation of a myasthenic patient is occluded, there is observed a progressive decline in the size of the muscle action potentials in response to nerve stimulation. In one experiment, for example, the voltage of the action potentials fell from an original value of 10.3 millivolts at the beginning of occlusion to 8.4 millivolts after the circulation had been obstructed for 5 minutes. The change in voltage was not accompanied by a change in the duration of the action potential. The significance of this abnormality is not apparent.

Response to Pairs of Stimuli

When two stimuli, separated by varying intervals are applied in the normal individual, little difference between the two responses is observed (7). In the myasthenic patient, the response to the second stimulus is often smaller than the response to the first. It appears that the response of the myasthenic muscle to a single stimulus is followed by a long period of depression during which a second stimulus fails to evoke a maximum response (fig. 1). Thus in one experiment, when stimuli 20 milliseconds apart were applied, the action potential following the second was only 96 per cent of that following the first; at an interval of 35 milliseconds, the second was 90 per cent of the first; while at an interval of 80 milliseconds, it was only 81 per cent.

The length of this period of depression is evidenced by the fact that 2 seconds after an initial stimulus, a second response may still be of

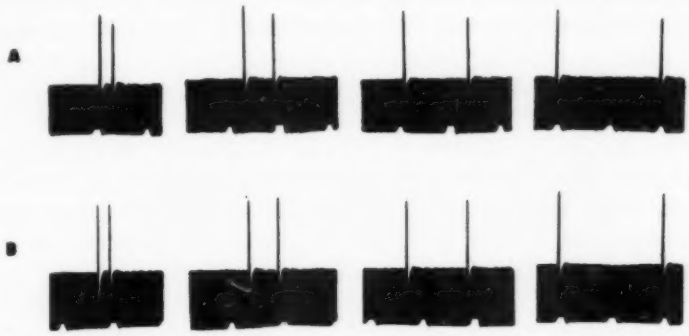

FIG. 1. Muscle action potentials in response to maximal stimulation of the ulnar nerve in a patient with myasthenia gravis. (A) Before prostigmin. (B) Thirty minutes after 1.0 mg. prostigmin. Time 0.2 sec. Initial potential 6 millivolts.

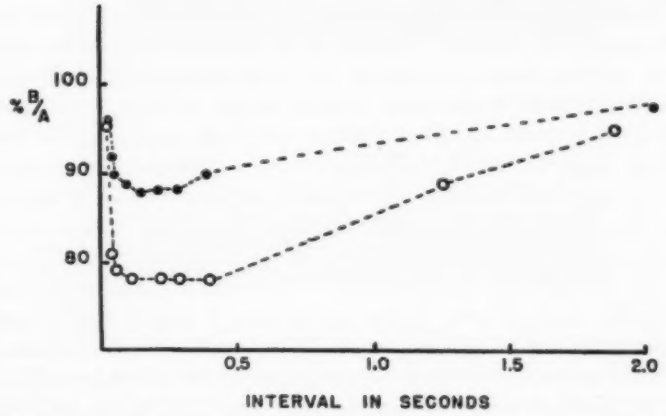

FIG. 2. Recovery curve of the second response of a pair. The voltage of the second response of a pair, expressed as percentage of the first, is plotted against the interval between the two stimuli. Solid dots, myasthenia gravis. Circles, normal subject after partial curarization with quinine methochloride.

reduced size. The depression of the second of two contractions has a time course similar to that observed in normal individuals who have been partially curarized by means of quinine methochloride (fig. 2).

This depression, seen as a result of activity in the myasthenic muscle, is not due to an increase in the temporal dispersion of the fibre responses under these conditions, for the duration of the reduced potential is shorter than that of the initial response. Thus, for example, the duration of the first response of a pair at a 95-millisecond interval was 14 milliseconds, while that of the second response was 12 milliseconds.

The decrease in duration of the response parallels the decrease in its voltage, a fact which indicates that both are caused by a reduction in the number of muscle fibers responding. It is unlikely that such a change could be due to temporal dispersion, or to changes in the individual potentials of the muscle fibers themselves.

Repetitive stimulation

When the nerve is stimulated with a train of impulses at frequencies up to 50 per second, the muscle action potentials of normal individuals show little change in size or character during the resulting tetanus. At frequencies of stimulation above 60 per second, there is usually a slow, progressive decline in the size of the response (7). In the myasthenic patient, however, even at low frequencies of stimulation, the muscle action potentials show a rapid decline in voltage during the first few responses, and then continue with little change at this lower level of response (fig. 3A). The decline is more pronounced when the muscle is partially fatigued as a result of previous periods of stimulation (fig. 3B). The degree of the fall in potential depends on the frequency of the tetanus. At a frequency of stimulation of 13 per second, for example, the potential of the tenth response of the train had fallen to 75 per cent of that of the first; at 43 per second, it was 40 per cent; and at 60 per second, 20 per cent. As in the case of pairs of responses, the decrease in amplitude of the action potentials is not the result of temporal dispersion, for again the action potentials show a decrease in duration as well as in amplitude.

In order to determine the time course of recovery following stimulation, a standard tetanus was given and was followed at varying intervals by a single test shock. Ample time was allowed between tetani to permit full recovery. From these results, the curve shown in figure 4 was constructed. The action potential does not regain its

initial voltage until 1500 milliseconds after the termination of the tetanus.

This characteristic decline in amplitude of successive potentials of a train has been observed only in patients with myasthenia gravis, and

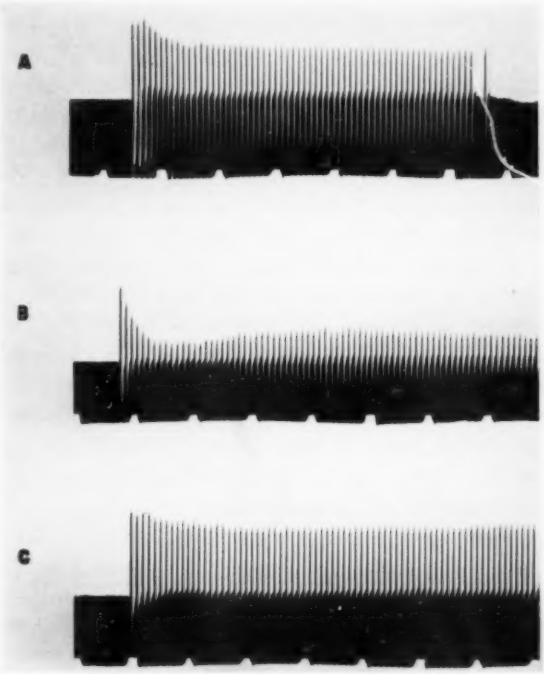

FIG. 3. Muscle action potentials during repetitive stimulation of the ulnar nerve, in a patient with myasthenia gravis. (A) Initial tetanus after rest. (B) Second tetanus ten seconds after the first. (C) Same as (B) thirty minutes after prostigmin. All nerve stimuli supramaximal. Time 0.2 sec. Initial potential 6.0 millivolts.

in normal individuals after the administration of curarizing drugs. Patients with other conditions, such as hyperthyroidism, muscular atrophy, amyotrophic lateral sclerosis and myotonic dystrophy, in which muscular weakness is a prominent symptom, do not show the characteristic reaction. The abnormality is of such specificity that

we suggest that its presence is diagnostic for myasthenia gravis or similar conditions.

Effect of Prostigmin

Twenty to thirty minutes after the subcutaneous administration of 1.5 mg. of prostigmin, the abnormality in the electromyogram of myasthenic patients was completely eliminated. When pairs of stimuli were given, the muscle action potential in response to the second stimulus was identical with that after the first (fig. 1B). Similarly there was no falling off in the potentials during tetanus. Even at a frequency as high as 65 per second, the potentials were indistinguishable from the normal (fig. 3C). It is possible that in patients suffering from severe forms of the disease, the administration of prostigmin may not be capable of causing the action potential to return to normal. In the patients examined, whose disease was in a mild stage, recovery appeared to be complete. The voltage of the action potential in response to a single supramaximal nerve volley was not significantly altered by the administration of prostigmin. This may be interpreted as indicating that in these patients, failure of conduction occurs only on repeated stimulation. Patients with more severe involvement of the musculature of the extremities should show a reduction in the voltage of a single potential and this should increase when the degree of neuromuscular block is decreased by prostigmin.

Effect of other drugs

Quinine, when given to myasthenic patients in two 0.6 gram doses, causes an increase in symptoms (10). When the electrical responses obtained following this form of medication are compared with those observed previous to it, the abnormalities are found to have been much increased.

Potassium chloride has been advocated for the treatment of myasthenia (11). Two patients were given from 6.0 to 8.0 grams of potassium chloride daily for a week. At the end of this time, there was slight subjective improvement, but the characteristic defects in the electromyogram remained unchanged. Similarly the administration of *guanidine* appeared to produce some subjective improvement, but did not change the electrical response of the muscle. The intravenous

administration of 20 to 30 mgm. per kgm. failed to produce any change in the action potentials over the ensuing three hours. *Calcium chloride*, 0.5 to 1.0 gram intravenously, also produced no detectable change.

The intravenous administration of *vitamin* B_6 was also tested. Fifty mgm. of this substance were given daily for three consecutive days. The electrical responses of the muscle failed to show any improvement, but continued to have the abnormality characteristic of the disease. In addition, contrary to the report of Spies (1938) (12), we have failed to note any improvement in strength following the use of this substance in myasthenic patients.

Thus, of all the substances which are said to be beneficial in the treatment of myasthenia gravis, prostigmin is the only one tested which, when given in ordinary dosage, is capable of decreasing the abnormality seen in the action potential of the muscle.

DISCUSSION

We are unable to account for the discrepancies between our results and those of Eichler and his coworkers (8). There are no resemblances between the abnormal electrical phenomena seen in myasthenia and those which develop following prolonged activity in the normal individual. The findings of Lindsley (9), using concentric needle electrodes, are in accord with the experimental results which have been presented in this paper, but the method which he used is not reliable for quantitative study. He records an increase in the mechanical response of myasthenic muscles to a single nerve shock following the administration of prostigmin, while we found no appreciable change in the size of the action potential. This discrepancy may be the result of one of two factors: the degree of neuro-muscular block to the passage of a single nerve impulse was greater in Lindsley's patients than in ours, and thus may have shown a greater change following the use of prostigmin; or, he may merely have recorded the potentiation of the twitch which occurs due to repetitive discharge following a single shock after prostigmin.

Pritchard's observation that there is no change in the mechanical myogram in response to repetitive stimulation until a frequency of 80 per second is reached is not in agreement with our studies using the action potential as an indication of muscle activity. The typical

decline in potential occurs with frequencies of stimulation as low as 12 per second. Even normal individuals show a slight decrease in the voltage of their muscle action potentials when tetanized at frequencies above 60 to 70 per second.

The striking similarity of the manifestations which result from partial curarization to the symptoms of myasthenia gravis, first pointed out by Oppenheim (13), has been emphasized by all who have witnessed the action of curarizing substances. In myasthenia gravis, the earliest signs almost always appear in the muscles innervated by the cranial nerves. There is weakness of the extrinsic eye muscle, difficulty in chewing and swallowing, and weakness of the palate with nasal speech. In severe cases, the extremities also become weak. Following the intravenous administration of curare, erythroidin, or quinine methochloride, all of which are curarizing substances, symptoms develop in the same order. The clinical picture of the two conditions is indistinguishable.

Similarly, the characteristic abnormalities of the electrical responses of the myasthenic and the curarized muscle are identical in all respects (fig. 5).

This similarity of the clinical and electromyographic picture of myasthenia gravis to that of curarization affords a basis on which to formulate hypotheses as to the localization and the nature of the abnormality responsible for the specific type of muscular weakness found in the disease. In accord with this similarity is the nature of the drugs which have been found to improve muscle power in myasthenia gravis. Prostigmin, potassium, and guanidine are all antagonistic to curare in their action. It would be fallacious to base an hypothesis on either the similarity in the clinical picture, in the electrical response, or in the action of drugs, but when these three features are considered together, it would seem justifiable to speculate on the nature of myasthenia gravis in relation to its close resemblance to partial curarization.

Brown (14) has observed that in the cat, following small doses of curarine, there is a depression following activity which is similar to that which we have described in human subjects under conditions of curarization and in patients with myasthenia gravis. He has suggested that this long period of depression, which may last a full second after a single muscle response, is due to changes in the threshold to

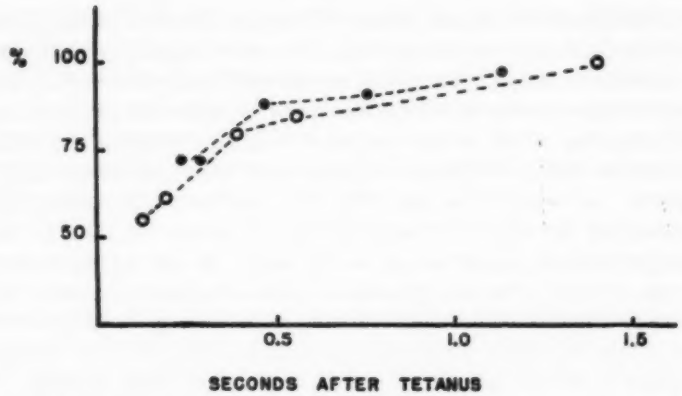

FIG. 4. Changes in the potential of test stimulus at varying intervals after a standard tetanus. Voltage, expressed as per cent of the voltage of the initial response of the tetanus, is plotted against interval after the tetanus. Circles, myasthenia gravis. Dots, normal subject after partial curarization with quinine methochloride.

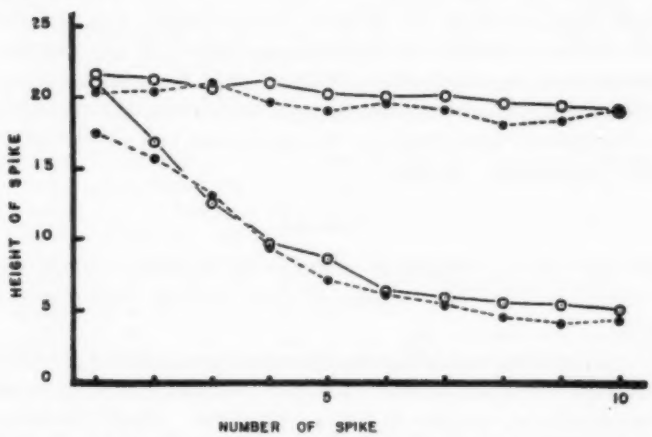

FIG. 5. Graph showing changes in the size of the successive responses during a tetanus. The size of the response in millimeters is plotted against the number of the response in the train. Solid dots show response of a normal subject before (upper curve) and after (lower curve) partial curarization with quinine methochloride. Circles show response of a myasthenic patient before (lower curve) and after (upper curve) prostigmin.

acetylcholine of the muscle fibres. When the threshold of the fibres becomes elevated by curarizing drugs, they are no longer able to respond to amounts of acetylcholine which are normally quite adequate. Because of this, a reduction in the amount of acetylcholine produced by each stimulus, which always occurs during the course of repeated stimulation, now is evidenced by progressive failure of the muscle to respond. As evidence for the belief that curare acts by raising the threshold of the muscle to acetylcholine, he points out that, in the curarized animal, injections of acetylcholine do not produce their normal response, whereas, injections of potassium salts continue to be normally effective (15). Furthermore, perfusion experiments reveal that in the curarized preparation stimulation still produces normal amounts of acetylcholine (16). On the basis of these findings, it appears that curarizing agents produce their characteristic neuromuscular block by altering the threshold of the muscle fibres so that they can not respond to the action of an impulse reaching the motor nerve ending.

As previously emphasized, the similarity of the clinical picture and the time course of the "fatigue" in curarization and myasthenia gravis makes a common mechanism seem likely. It suggests that in myasthenia as in curarization, the defect is in the muscle fibre or in its motor end plate, and that through this defect, the threshold of this structure to stimulation by the mechanism responsible for junctional transmission is elevated.

SUMMARY

(1) The electromyogram in response to maximal stimulation of the ulnar nerve has been studied in three patients with myasthenia gravis.

(2) A single response of the myasthenic muscle is followed by a prolonged depression during which a second maximal motor nerve volley excites a reduced number of the muscle fibres. When the nerve is stimulated with a train of impulses, the resulting muscle action potentials show a progressive decline in voltage.

(3) Prostigmin abolishes these abnormalities in the electromyogram of myasthenic patients, but potassium chloride, guanidine, calcium chloride, and vitamin B_6 have no effect.

(4) The abnormalities are increased by the administration of quinine.

(5) The characteristic changes in the electromyogram in myasthenia are similar to those seen as a result of partial neuromuscular block by curarizing substances.

(6) The nature of the abnormality in myasthenia gravis is discussed in relation to these findings.

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OBSERVATIONS ON THE MODE OF ACTION OF SULFANILAMIDE

The Antibacteriostatic Action of Methionine[1]

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From the Department of Preventive Medicine of The Johns Hopkins University Medical School

Submitted for publication May 9, 1941

Interest in the observation made by Lockwood (1) that the addition of peptone to an unfavorable medium tended to counteract the bacteriostatic action of sulfanilamide led us to undertake the present study. At first glance it seemed possible that the antagonistic effect of peptone toward sulfanilamide might be due to the much better growth obtained in the presence of peptone. However, the finding that although the addition of glucose to the unfavorable medium greatly enhanced growth it had no effect upon sulfanilamide's action, suggested that growth promotion and antisulfanilamide effect might not always go hand in hand. We, therefore, decided to determine what other substances might exhibit a similar antisulfanilamide action, in the hope that such a study would throw some light on the mechanism of action of the sulfonamide compounds.

The fact that one of the essential amino acids neutralized the action of sulfanilamide was reported by us over a year ago (2). The present paper is a full account of the experiments which led up to that observation.

METHODS

In order to obtain the most exact conditions possible for this study it was decided to employ a synthetic medium, all the constituents of which were well defined chemical substances. The organism selected was E. coli.

Media

Four basic media were used (see Table I). Two were plain salt bases (SB1 and SB4) and contained no source of nitrogen. E. coli grew poorly in these

[1] This work has been supported by a grant from the John and Mary R. Markle Foundation, New York City.

[2] Calco Division, American Cyanamid Company, Fellow in Preventive Medicine.

unless nitrogenous matter was added. SB5 contained the same salts as SB4 plus ammonium citrate. When dextrose was added and a large inoculum (100,000 bacteria) used, good growth occurred in 24 hours, and inocula of 1000 bacteria grew out to 500 million in 48 hours. The S1 media contained asparagin as well as ammonium citrate. Growth occurred even in the absence of dextrose, and from small inocula, but it required 5 days for it to reach a maximum. In the presence of 0.1 per cent dextrose inocula of 50 bacteria per cc. grew out to about 900 million in 24 hours at 37°C. With smaller amounts of dextrose, growth was slower. Larger amounts, up to 2 per cent, did not materially increase the rate or abundance of growth.

Preparation of Media

The constituents were added to distilled water and brought to the boiling point. The solution was then distributed in smaller flasks and autoclaved at 15 pounds for 15 minutes. With SB4 it was found necessary to withhold the magnesium

TABLE I

Constituents of Basic Media in Grams per cent

DESIGNATION	NaCl	K_2HPO_4	KH_2PO_4	$MgSO_4, 7H_2O$	AMMONIUM CITRATE	ASPARAGIN	NaOH (OR Na_2CO_3)
SB1	0.5	0.2	0.06	0	0	0	Not needed
SB4	0.5	0.2	0.06	0.05	0	0	" "
SB5	0.5	0.2	0.06	0.05	0.2	0	To pH 7.3
S1	0.5	0.2	0	0.05	0.2	0.35	To pH 7.3
S1G1	Same as S1 with 0.1% dextrose added						

sulphate until after autoclaving, adding it from a sterile solution. Otherwise precipitation occurred. (In the presence of ammonium citrate there was no precipitation.)

Strains

Three strains of E. coli were employed. "Coli" and "Smith" were of the communior type while "Jordan" was E. coli communis. All three were isolated from the urine of patients ill with pyelitis. The majority of tests were run with "Coli," "Smith" and "Jordan" being introduced toward the end of the study only to see if the results obtained with "Coli" could also be obtained with other strains of escherichia.

Procedure

The test substances were added to one or another of the basic media in the desired concentration. Where necessary the solutions were sterilized by boiling for half a minute or so. They were then pipetted, aseptically, into sterile Wasser-

mann tubes in such quantities as would result in a final volume of 5 cc. The test organism, grown for 16 to 20 hours in S1G1, was diluted either in the same basic medium as that used in the test or in SB1 or SB4. Dilution was carried to 10^{-6} and 0.1 cc. of this dilution constituted the inoculum. A poured plate was made with the same amount in order to determine how many bacteria had been inoculated. The inoculum ranged from 70 to 200 bacteria, depending more or less upon the age of the original culture. The cultures were incubated at 37°C. for 5 days as a rule. The growth was observed at 24, 48, 72, and 120 hours and recorded with plus and minus symbols. Plates were usually poured at 24 hours from suitable dilutions in beef infusion broth. Occasionally, they were also made at later periods.

RESULTS

Growth and Bacteriostasis in the S1 Media

The growth of the 3 strains of E. coli in the two S1 media and the effect upon it of various concentrations of sulfanilamide, sulfapyridine, and sulfathiazole are shown in Table II. It is unnecessary to comment on this table except to call attention to the marked bacteriostatic effect of 10 mgm. per cent of sulfanilamide upon the 3 organisms in these media. This is to be contrasted with the poor inhibition of E. coli noted with concentrations of 200 mgm. per cent of sulfanilamide in broth. It is also of interest that very low concentrations of sulfapyridine and sulfathiazole must be used in order to bring out the difference in the bacteriostatic action of these two compounds.

When other carbohydrates (glycerol, arabinose, rhamnose, xylose, galactose, levulose, lactose, maltose, saccharose, raffinose, dulcitol, mannitol, and salicin) were used in the place of dextrose as good bacteriostasis was obtained as in the S1G1 medium. Many of these carbohydrates, however, did not enhance the growth over that obtained in the sugar free S1 medium.

The Effect of Adding Various Nitrogenous Substances to the Basic Media

Attention was next turned to nitrogenous compounds. These were studied in the order of their complexity starting with serum, albumin and gelatin, and continuing down through peptone and biuret-free casein hydrolysates, to amino acids and urea. Yeast nucleic acid was also tested. The effect of these substances upon the bacteriostatic action of sulfanilamide is shown in Table III.

TABLE II

The Bacteriostatic Action of Sulfanilamide, Sulfapyridine, and Sulfathiazole upon the Growth of 3 Strains of E. coli in Synthetic Media

MEDIUM	"COLI"				"JORDAN"				"SMITH"			
	24	48	72	120	24	48	72	120	24	48	72	120
SI	±10.5M§	+	+++	++++								
SI + $PABS_{10}$*	−86T	−	−	−								
SIGI	++++ 935M	+++++ 1.4B	+++++ 1.8B	+++++ 900M	++++ 774M	++++	+++++ 1.55B	+++++	++++ 721M	++++	+++++ 1.6B	+++++
SIGI + $PABS_{10}$	−82T	−200T	−500T	+ & −¶ 19M	−−	−−	−−	−−	−−	−−	−−	−−
$PABS_1$	+18.8M	++++	+++++	+++++	+19M	++++	++++	+++++	+71M	++++	+++++	+++++
SP_{10}†	−1.2T	−	−	−	−	−	−	−	−5.3T	−	−	−
SP_5	−1.8T	−	−	−	−5.7T	−	−	−	−7.5T	−	−	−
SP_1	−2T	−	−29T	−	−7.5T	−	−73T	−	−22.6T	−	−53.6T	−
$SP_{0.5}$	−2M	±	±	++++	−1.5M	±	±	+++++	−90T	−	−	++++
ST_{10}‡	−900	−	−	−	−10T	−	−	−	−3.6T	−	−	−
ST_5	−1.1T	−	−	−	−7T	−	−	−	−5.9T	−	−	−
ST_1	−1.8T	−	−700	−	−3.8T	−	−4.3T	−	−4.6T	−	−10.2T	−
$ST_{0.5}$	−4.8T	−	−	−	−60T	−	−	−	−13T	−	−	++
$ST_{0.1}$	−206T	−	−	++++								

* $PABS_{10}$ = 10 mgm.% sulfanilamide.

† SP = Sulfapyridine—SP_{10} = 10 mgm.% concentration of sulfapyridine.

‡ ST = Sulfathiazole—ST_{10} = 10 mgm.% concentration of sulfathiazole.

§ B = billion; M = million; T = thousand.

¶ Usually no visible growth.

It will be observed that serum, albumin, and gelatin interfered but slightly with the activity of the drug as compared with the effect of

TABLE III-a

The Effect of Adding Various Nitrogenous Substances to Basic Media upon the Bacteriostatic Action of Sulfanilamide (10 mgm. %) on "Coli"

A. Serum

BASE	SERUM	CONC. (%)	PABB 10	GROWTH—HOURS AFTER INOCULATION			
				24	48	72	120
S1	Horse	1.0	−	+++ 350M	+++ 500M	++++ 800M	+++++ 530M
		1.0	+	− <10,000	− 193,000	− 400,000	− to + 23M
		0.1	−	+ 15M	++	++++ 280M	+++++
		0.1	+	− <10,000	−	− 42,000	− to ±
		0.01	−	±	+	+++	++++
		0.01	+	−	−	−	−
	Human	1.0	−	++ 130M	++++ 480M	+++++	+++++
		1.0	+	− 1.4M	+ 8M	+	+
		0.1	−	+	+++	+++++ 1.3B	+++++ 950M
		0.1	+	−	−	± 1.5M	− 700,000
		0.01	−	−	−	−	±
		0.01	+	−	−	−	−
	Rabbit	1.0	−	+++ 270M	++++ 1.08B	+++++	+++++
		1.0	+	− 4.1M	± 750,000	+	++
		0.1	−	+	+++	+++++ 1.4B	+++++ 1.2B
		0.1	+	−	−	− 920,000	+ 1.7M
		0.01	−	−	−	−	−
		0.01	+	−	−	−	−
SB_1	Horse	1.0	−	− 150,000	±	+ 10M	+ 44M
		1.0	+	− 1,800	−	± 20M	+ 13M
		0.1	−	−	−	−	−
		0.1	+	−	−	−	−
		0.01	−	−	−	−	−
		0.01	+	−	−	−	−
	Human	1.0	−	±	±	+ 80M	+
		1.0	+	−	±	+ 30M	+
		0.1	−	−	±	± 20M	± 29M
		0.1	+	−	−	− 300,000	− 20,000
		0.01	−	−	−	−	−
		0.01	+	−	−	−	−
	Rabbit	1.0	−	± 22M	±	+ 70M	+ 69M
		1.0	+	− 40,000	−	− 400,000	± 800,000
		0.1	−	−	−	−	−
		0.1	+	−	−	−	−
		0.01	−	−	−	−	−
		0.01	+	−	−	−	−

peptone. Further split products of protein, as exemplified by the biuret-free casein hydrolysates had very much the same effect as

peptone, but yeast nucleic acid and urea appear to have been toxic in the higher concentration and exhibited no antisulfanilamide action.

TABLE III-b

The Effect of Adding Various Nitrogenous Substances to Basic Media upon the Bacteriostatic Action of Sulfanilamide (10 mgm.%) on "Coli"

B. Albumin, Gelatin

BASE	N COMPOUND	CONC. (%)	PABB 10	GROWTH—HOURS AFTER INOCULATION							
				24		48		72		120	
S1	Egg albumin	1.0	−	+++	760M	++++		++++			
	(Merck-powdered)	1.0	+	+	89M	++++		++++			
		0.1	−	++	224M	+++		++++			
		0.1	+	−	100,000	±		+			
	(Fresh)	1.0	−	++		+++	390M	++++		++++	
		1.0	+	−		±	8M	+		±	
		0.1	−	+		++		+++		++++	1,760M
		0.1	+	−		−		−		−	50,000
		0.01	−	−		−		+		++++	
		0.01	+	−		−		−		−	
SB1	(Fresh)	1.0	−	−		±		+	10M	+	
		1.0	+	−		−		±	50,000	±	
		0.1	−	−		−		±		±	1M
		0.1	+	−		−		−		−	300,000
		0.01	−	−		−		−		−	
		0.01	+	−		−		−		−	
S1	Gelatin	1.0	−	+	110M	+++		++++	1.0B	++++	
		1.0	+	+	90M	++		++	286M	+++	
		0.1	−	+	40M	+		+++	230M	++++	
		0.1	+	−	2.8M	−		−	4.8M	±	
		0.01	−	−		+		++		++++	
		0.01	+	−		−		−		−	
S1G1	"	1.0	−	++++		++++		++++		++++	
		1.0	+	+		+++		++++		++++	
		0.1	−	++++		++++		++++		++++	
		0.1	+	−		−		−		−	
		0.01	−	+++		++++		++++		++++	
		0.01	+	−		−		−		−	
SB1	"	1.0	−	−	<100,000	−		±		±	
		1.0	+	±	500,000	±		±		±	
		0.1	−	−		−		−		−	
		0.1	+	−		−		−		−	
		0.01	−	−		−		−		−	
		0.01	+	−		−		−		−	

The Effect of Amino Acids Upon Bacteriostasis

Study of the effect of amino acids upon the bacteriostatic action of sulfanilamide presented a problem more complex than the preced-

TABLE III-c

The Effect of Adding Various Nitrogenous Substances to Basic Media upon the Bacteriostatic Action of Sulfanilamide (10 mgm. %) on "Coli"

C. Peptone

BASIC MEDIUM	NITROGEN COMPOUND	CONC. (%)	PABB 10	GROWTH—HOURS AFTER INOCULATION			
				24	48	72	120
SIGI	—	0	−	++++ 300M	++++	++++	++++
	—	0	+	− 100,000	−	−	−
	Neopeptone	0.1	−	+++++ 1B	++++	++++	++++
		0.1	+	++++ 600M	++++	++++	++++
		0.01	−	++++ 300M	++++	++++	++++
		0.01	+	− 170,000	+	+++	++++
		0.001	−	++++ 300M			
		0.001	+	− 10,000	±		
		1.0	−	++++	++++	++++	++++
		1.0	+	++++	++++	++++	++++
SI	—	0	−	± 10.5M	+	+++	++++
	—	0	+	− 8600	−	−	−
	Neopeptone	1.0	−	+++	++++	++++	++++
		1.0	+	+++	++++	++++	++++
		0.1	−	+++ 500M	++++	++++	++++
		0.1	+	++ 94M	++	++	++
		0.01	−	++ 140M	++	+++	++++
		0.01	+	++ 81M	+	+	+
		0.001	−	+ 25M			
		0.001	+	± 1.4M			
	Tryptose peptone	0.1	−	+++ 250M	++++	++++	
		0.1	+	++ 120M	+++	+++	
	Bacto peptone	0.1	−	+++ 340M	++++	++++	
		0.1	+	++ 90M	+++	+++	
	Proteose peptone	0.1	−	++++ 650M	++++	++++	
		0.1	+	++ 50M	++	+++	
SB_1	Neopeptone	1.0	−	++	++++	++++	++++
		1.0	+	++	++++	++++	++++
		0.1	−	+ 244M	++	++	++
		0.1	+	+ 190M	++	++	++
		0.01	−	±	±	±	±
		0.01	+	±	±	±	±
	Tryptose peptone	1.0	−	+++	+++++	+++++	+++++
		1.0	+	+++	++++	++++	+++++
		0.1	−	+ 454M	++	++	+++
		0.1	+	+ 694M	++	++	+++
		0.01	−	−	±	+ 256M	+
		0.01	+	−	−	− 141,000	−
	Proteose peptone	1.0	−	+++	+++++	+++++	+++++
		1.0	+	+++	++++	++++	++++
		0.1	−	+++ 553M	++	++	+++
		0.1	+	++ 397M	++	++	+++
		0.01	−	±	±	±	±
		0.01	+	±	±	±	±

TABLE III-d

The Effect of Adding Various Nitrogenous Substances to Basic Media upon the Bacteriostatic Action of Sulfanilamide (10 mgm. %) on "Coli"

D. Biuret-free Protein Hydrolysates

BASE	N COMPOUND	% CONC.	PASS 10	GROWTH—HOURS AFTER INOCULATION							
				24		48		72		120	
S1	Difco* #1	1.0	−	++	350M	++++		++++			
		1.0	+	+	144M	++		++++			
	#2	1.0	−	+++	330M	++++		++++			
		1.0	+	+	77M	++		++++			
	#3	1.0	−	++	250M	++++		++++			
		1.0	+	++	193M	+++		+++			
	#4	1.0	−	+++	500M	++++		+++++			
		1.0	+	+	60M	++		++++			
	Arlco† Milk	1.0	−	++++	630M	++++		+++++			
		1.0	+	++++	660M	++++		+++++			
	Beef	1.0	−	++++	1.5B	++++		+++++			
		1.0	+	++++	940M	++++		+++++			
SB_1	Difco #1	1.0	−	++	470M	++++		+++++		+++++	
		1.0	+	+	163M	++		+++		++++	
		0.1	−	−		−		−		−	
		0.1	+	−		−		−		−	
		0.01	−	−		−		−		−	
		0.01	+	−		−		−		−	
	#2	1.0	−	+++	750M	++++		+++++		+++++	
		1.0	+	+	117M	+++		++++		++++	
		0.1	−	−		++	1.7B	+++		+++	
		0.1	+	−		−	1.7M	++		++	
		0.01	−								
		0.01	+	−		−		−		−	
	#3	1.0	−	++	170M	+++		++++		+++++	
		1.0	+	+	109M	++		+++		++++	
		0.1	−	−		−		−		−	
		0.1	+	−		−		−		−	
		0.01	−	−		−		−		−	
		0.01	+	−		−		−		−	
	#4	1.0	−	+++	1.07B	++++		+++++		+++++	
		1.0	+	+	161M	+++		++++		++++	
		0.1	−	−		−		−		−	
		0.1	+	−		−		+		++	
		0.01	−	−		−		−		−	
		0.01	+	−		−		−		−	
SB_4	#4	1.0	−	++	188M	++++					
		1.0	+	−	500,000	++					
SB_4 + dex. 0.1	#4	1.0	−	++++	1.5B	+++++					
		1.0	+	−	3.0M	++++					

* The Difco preparations were labeled: Casein hydrolysate S-12394 #1, 2, 3, and 4.

† The Arlco preparations were labeled as follows:
Arlco Peptone Biuret Free (Milk) Total Nitrogen 10.44%, Amino N 49.53% of total.
Arlco Peptone Biuret Free (Beef) Total Nitrogen 9.39%, Amino N 57.56% of total.

ing one, and it was entered into in more detail. The amino acids selected for investigation were the ten which are known to be essential

TABLE III-e

The Effect of Adding Various Nitrogenous Substances to Basic Media upon the Bacteriostatic Action of Sulfanilamide (10 mgm. %) on "Coli"

E. Urea and Yeast Nucleic Acid

BASE	N COMP.	% CONC.	PABS 10	GROWTH—HOURS AFTER INOCULATION			
				24	48	72	120
SI	—	0	−	±	+	++	++++
		0	+	−	−	−	−
	Urea	1.0	−	−	±	+	+++
		1.0	+	−	−	−	−
		0.1	−	±	+	++	++++
		0.1	+	−	−	−	−
		0.01	−	±	+	++	++++
		0.01	+	−	−	−	−
SIGI	—	0	−	+++	++++	++++	++++
		0	+	−	−	−	−
	Urea	1.0	−	±	+++	++++	++++
		1.0	+	−	−	−	−
		0.1	−	++	++++	++++	++++
		0.1	+	−	−	−	−
		0.01	−	+++	++++	++++	++++
		0.01	+	−	−	−	−
SI	Yeast nucleic acid	1.0	−	±	±	±	±
		1.0	+	−	−	−	−
		0.1	−	±	+	++	+++
		0.1	+	−	−	−	−
		0.01	−	±	+	++	++++
		0.01	+	−	−	−	−
SIGI	Yeast nucleic acid	1.0	−	−	−	−	−
		1.0	+	−	−	−	−
		0.1	−	±	+++	++++	++++
		0.1	+	−	−	−	−
		0.01	−	+++	++++	++++	++++
		0.01	+	−	−	−	−

for animal metabolism; namely, arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophane and valine.

It was considered desirable to use synthetic products and for this reason the d-l derivatives were acquired as far as possible. We were able to get the d-l forms[3] of isoleucine, leucine, lysine dihydrochloride, methionine, phenylalanine, threonine, and valine. The other amino acids were d-arginine monohydrochloride[4], l-histidine monohydrochloride[5] and l-tryptophane.[6]

None of the amino acids, when added separately in 0.2 per cent concentration to SB4 (plus 0.1 per cent dextrose), gave good growth, but five did produce a scanty growth at the end of seven days. It seemed at first, therefore, that it would be necessary to study these substances in combination.

Bacteriostasis in the Presence of Combinations of Amino Acids

When all ten amino acids were added in 100 mgm. per cent concentration to SB4 or SB5 very good growth and very little bacteriostasis from sulfanilamide was obtained (Table IV). Two combinations of 5 amino acids were then prepared, approximately 200 mgm. per cent of each amino acid being used. The first combination was composed of the amino acids which had shown a slight ability to promote growth in SB4; these were arginine, isoleucine, leucine, methionine, and tryptophane (Group A). This combination resembled the combination of 10 amino acids in its ability to support growth and in its antisulfanilamide action. In the second combination, containing the other amino acids (Group B), "Coli" failed to multiply during five days of incubation. It was, therefore, decided to restrict further study of the amino acids to those in Group A for the time being.

For combinations of less than five amino acids, it was deemed advisable to prepare them in solutions of such concentrations that equal volumes of each would provide equal amounts of nitrogen. The desired total nitrogen content of the final solution was 154 mgm. per cent—the amount found on analysis to be present in the medium containing Difco casein hydrolysate #4 in SB5. Solutions in SB4 were therefore made up as follows: Arginine 0.9 per cent, isoleucine 1.4

[3] From the Department of Chemistry, The University of Illinois, Urbana, Illinois.

[4] The Mearl Corporation, 153 Waverly Place, New York City.

[5] The Fordomes Trading Company, 21 Lafayette Place, Trenton, New Jersey.

per cent, leucine 1.4 per cent, methionine 1.6 per cent, tryptophane 1.1 per cent, and for combinations of 4 amino acids, for example, 1.25 cc. of each solution was used.

TABLE IV

Growth of Coli and Bacteriostasis by Sulfanilamide (10 mgm. %) in Various Combinations of Amino Acids in SB_3 or SB_4 (with Dextrose)

10, 5, and 4 amino acids

AMINO ACIDS	BASE (+ 0.1% DEXTROSE)	PABS 10	GROWTH—HOURS AFTER INOCULATION				
			24		48	72	120
AA_{10}*	SB_4	−	+++	524M§	+++	++++	++++
		+	+++	608M	+++	++++	++++
AA_{10}	SB_3	−	+++	516M	++++	++++	+++++
		+	+	188M	++++	++++	+++++
AA_{in}†	SB_4	−	+++	561M	+++	++++	++++
		+	+++	488M	+++	++++	++++
AA_{in}	SB_3	−	++++	925M	++++	++++	++++
		+	++	286M	++++	++++	++++
AA_{sb}‡	SB_3	−	−	1,500	−	−	−
Arginine, isoleucine, leucine, and methionine	SB_4	−	−	1.1M	++	++	+++
		+	−	1.6M	++	+++	+++
Arginine, isoleucine, leucine, and tryptophane	SB_4	−	+++	360M	+++	+++	+++
		+	±	4.7M	±	±	±
Arginine, isoleucine, methionine, and tryptophane	SB_4	−	+++	400M	+++	+++	+++
		+	+++	370M	+++	+++	+++
Arginine, leucine, methionine, and tryptophane	SB_4	−	+++	390M	+++	+++	++++
		+	+++	370M	+++	+++	++++
Isoleucine, leucine, methionine, and tryptophane	SB_4	−	±	1M	++	++	+++
		+	−	10,000	±	++	+++

* AA_{10} = 100 mgm. % of each of the 10 amino acids.

†AA_{in} = 218 mgm. % each of arginine, isoleucine, leucine, methionine, and tryptophane.

‡AA_{sb} = 195 mgm. % each of histidine, lysine, phenylalanine, threonine, and valine.

§ The figures represent the averages of two or more experiments.

There were five combinations each containing four amino acids. In two of these growth was poor at 24 hours, but increased as time went on. In both of them bacteriostasis by 10 mgm. per cent sulfanilamide was negligible. Growth in the other three combinations was excellent and in two of them again there was little or no bacteriostasis. In the remaining combination, however, while "Coli" grew out in the presence of sulfanilamide to 4 million per cc. in the first 24 hours, multiplication apparently stopped at that time. The amino acid which was absent from this combination was methionine.

The ten combinations containing three amino acids were studied next (Table V). Six of these contained methionine, four lacked it. Of the latter only one (#5) showed permanent bacteriostasis on both occasions in which it was tested and thus resembled the combination of four amino acids which lacked methionine. In two (#1 and #8) bacteriostasis was maintained through the whole period in only one of the two tests and in the fourth (#3) bacteriostasis was slight from the start on both occasions.

Of the six combinations in which methionine was present, there was good growth in only one (#6, arginine, methionine, and tryptophane), and here there was negligible bacteriostasis (two and three fold). In the other five combinations "Coli" grew poorly. In two of them (#9 and #10, isoleucine or leucine, methionine and tryptophane) there was good bacteriostasis which was maintained for the 5 day period. In the other three (#2, #4, and #7), however, there was better growth in the presence of sulfanilamide than in the control and in two the control never did grow out, while the cultures containing the drug finally reached a two to three plus turbidity.

Arginine plus tryptophane was the only combination of two amino acids which was tested because it appeared from the results already noted that good growth occurred only when both of these substances were present. In the first test (Table V) there was but little bacteriostasis which quickly disappeared. Later, however, 0.5 cc. each of the arginine and tryptophane solutions added to 4 cc. of S1G1 did not inhibit the antibacterial action of sulfanilamide (Table VII). A summary of the results with combinations of amino acids is shown in Table VI. There, in the last column, is shown whether or not the results were compatible with the original observation that methionine and it alone of the amino acids which were tested inhibited the action

TABLE V

Growth of Coli and Bacteriostasis by Sulfanilamide (10 mgm. %) in Various Combinations of Amino Acids in SB_4 (with Dextrose)

3 and 2 Amino Acids

AMINO ACIDS	PABA 10	GROWTH—HOURS AFTER INOCULATION			
		24	48	72	120
1. Arginine, isoleucine, leucine	−	± 86M	+++	+++	+++
	+	− 22,000	−	−	+++, −
2 Arginine, isoleucine, methionine	−	± 4M	++	+++	++++
	+	± 12M	++	+++	++++
3 Arginine, isoleucine, tryptophane	−	+++ 650M	++++	++++	+++
	+	± 10.2M	+	+++	+++
4 Arginine, leucine, methionine	−	− <1,000	− <1,000	− 10	− 0
	+	± 1M	+ 210M	++ 400M	+++ 600M
5 Arginine, leucine, tryptophane	−	+++ 545M	++++	++++	++++
	+	− 5.5M	−	−	−
6 Arginine, methionine, tryptophane	−	++ 215M	+++	+++	++++
	+	+ 80M	++	+++	++++
7 Isoleucine, leucine, methionine	−	− <1,000	−	−	−
	+	− 40,000	−	±	++
8 Isoleucine, leucine, tryptophane	−	++ 230M	+++	+++	+++
	+	− 560,000	±, −	+, −	+++, −
9 Isoleucine, methionine, tryptophane	−	± 18M	+	++	+++
	+	− 1,000	−	−	+++, −
10 Leucine, methionine, tryptophane	−	± 1M	+	++	+++
	+	− 1,000	−	−	−
11 Arginine, and tryptophane	−	++ 330M	+++	++++	++++
	+	+ 6.5M	+++	+++	++++

of sulfanilamide. Twenty-four of the thirty-three tests are in accord with that observation, but nine run counter to it. Most of the di-

vergent results are among the combinations which lacked methionine and where bacteriostasis was expected but ultimate growth occurred. This suggested that some other amino acid might be capable of counteracting bacteriostasis by sulfanilamide. To prove this possibility, tests with single amino acids were necessary and because "Coli" did not grow well in salt bases containing single amino acids it was decided that S1G1 had best be used. This medium, it will be remembered, contains asparagin as well as ammonium citrate.

Bacteriostasis in the Presence of Single Amino Acids

In the first experiment of this sort, 1 cc. of the amino acid solution (see p. 23) was added to 4 cc. of S1G1. The results are shown in Table VII. This experiment was repeated with all ten of the amino acids but on this occasion the same amount of each amino acid was used, i.e., an 0.5 per cent concentration. The results are shown in Table VIII. In both of these experiments, methionine was the only one of the amino acids tested which showed an action antagonistic to sulfanilamide. At a still later date, however, when methionine, arginine, leucine, lysine, and tryptophane were tested in 200 mgm. per cent concentrations in S1G1, arginine and lysine as well as methionine exhibited some antibacteriostatic activity. Manifestation of this property by arginine and lysine occurred 2 and 3 days after methionine had shown it. Since one occasionally sees outgrowth after 5 days in plain S1G1 plus 10 mgm. per cent sulfanilamide, it may be that the belated appearance of growth in the presence of arginine and lysine was not the result of any antisulfanilamide action. This point will be discussed later.

The Effect of Substances Related to Methionine Upon the Bacteriostatic Action of Sulfanilamide

Thinking that perhaps the sulphur in methionine might underlie the antisulfanilamide action of this amino acid, a number of other sulphur containing compounds were tested for antibacteriostatic activity. The substances selected were cysteine, homocystine[6], S-methyl cysteine[6], and homomethionine[6]. Cystine was not tested because of its low solubility. Homocystine, also only slightly soluble,

[6] Kindly supplied by Dr. Vincent du Vigneaud.

TABLE VI

Summary of Results with Combinations of Amino Acids

MIXTURE	GROWTH CONTROL, 24 HRS.	DEGREE OF‡ BACTERIOSTASIS AT 24 HRS.	BACTERIOSTASIS MAINTAINED FOR 5 DAYS	EXPECTED RESULT
Arginine, isoleucine, leucine	+	17,000	Yes	Yes
	−	2?	No	No
Isoleucine, leucine, tryptophane	++	900	Yes	Yes
	++	240	No	No
Arginine, isoleucine, leucine, tryptophane	+++	300	Yes	Yes
	+++	59	Yes	Yes
Arginine, leucine, tryptophane	++++	150	Yes	Yes
	+++	60	Yes	Yes
Arginine, isoleucine, tryptophane	+++	60	No	No
	++++	50	No	No
Arginine, tryptophane	++++	20	No	No
	+	5	No	No
Leucine, methionine, tryptophane	±	>15	Yes	No
	+	2,000	Yes	No
Isoleucine, methionine, tryptophane	+	300	Yes	No
	±	500	No	Yes
Isoleucine, leucine, methionine, tryptophane	+	100	No	Yes
	Control	Tube lost	No	Yes
Arginine, leucine, methionine, tryptophane	++++	Θ*	(No)†	Yes
	++	>1,600	No	Yes
AA_{10} in SB_8	+++	10, 8, 2½, 2, Θ	No	Yes
AA_5 in SB_8	++++	7, 4, 3½, 3, 2	No	Yes
Arginine, methionine, tryptophane	++	3	No	Yes
	++	2	No	Yes
AA_{10} in SB_4	+++	0, 0, 0, Θ, Θ	(No)	Yes
AA_5 in SB_4	+++	1, 8, 0, 0, 0, Θ, Θ, Θ	(No)	Yes
Arginine, isoleucine, methionine, tryptophane	++++	0	(No)	Yes
	++	Θ	(No)	Yes
Arginine, isoleucine, leucine, methionine	±	Θ	(No)	Yes
	+	Θ	(No)	Yes
Arginine, isoleucine, methionine	+	Θ Θ Θ	(No)	Yes
Arginine, leucine, methionine	−	Θ Θ Θ	(No)	Yes
Isoleucine, leucine, methionine	−	Θ Θ	(No)	Yes

* Θ = better growth in presence of PABS than in control.

† (No) = since there was no bacteriostasis at the start, it could hardly be maintained.

‡ Degree of bacteriostasis = ratio: $\frac{\text{growth in control}}{\text{growth in sulfanilamide}}$

was used in a saturated solution in S1G1. It exhibited no antisulfanilamide action. The other three substances, cysteine, S-methyl

cysteine and homomethionine, were toxic for E. coli when used in 200 mgm. per cent solutions, and there was no growth in either the presence or absence of sulfanilamide. A 4 mgm. per cent solution of homomethionine permitted growth but had no effect on sulfanilamide action. S-methyl cysteine was, again, toxic in this concentration but late growth occurred. Choline chloride[6]—200 mgm. per cent was also

TABLE VII

Effect of Adding Certain Amino Acids to S1G1 upon the Bacteriostatic Action of Sulfanilamide on Coli

Each amino acid supplying 35 mgm. % nitrogen

MEDIUM S1G1 *plus*	CONCENTRATION OF AMINO ACID	PABS 10	GROWTH—HOURS AFTER INOCULATION			
			24	48	72	120
	per cent					
—	0	−	++++ 1.2B	++++	++++	++++
		+	− <1,000	−	−	−
Arginine	0.19	−	++++ 800M	+++++	+++++	+++++
		+	− 200,000	−	−	−
Isoleucine	0.29	−	++++ 1.2B	+++++	+++++	++++
		+	− 21,000	−	−	−
Methionine	0.33	−	++++ 880M	+++++	+++++	+++++
		+	− 640,000	+	+++	++++
Tryptophane	0.22	−	++++ 870M	+++++	+++++	+++++
		+	− 20,000	−	−	−
Arginine and tryptophane	0.09	−	++++ 900M	++++	+++++	+++++
	0.11	+	− 710,000	−	−	−

tried in one experiment. Though it permitted excellent growth of coli, it did not interfere with the action of sulfanilamide.

The Effect of Increasing Concentrations of Methionine Upon the Antibacteriostatic Action of this Amino Acid

In Table IX it is shown that as little as 1 mgm. per cent of methionine interferes with the bacteriostatic action of 10 mgm. per cent of sulfanilamide, and that this interference is slightly increased with

larger amounts of methionine. At the same time methionine in concentrations of 1 per cent or greater appears at times to be toxic for

TABLE VIII

Effect of Adding Certain Amino Acids to S1G1 upon the Bacteriostatic Action of Sulfanilamide upon Coli

Same concentration of each amino acid

MEDIUM S1G1 *plus*	CONCENTRATION OF AMINO ACID	PASS 10	GROWTH—HOURS AFTER INOCULATION			
			24	48	72	120
	per cent					
—	0	+	++++ broken	+++++	+++++	+++++
		−	− 1,000	−	−	−
Arginine	0.5	+	++++ 1.31B	+++++	+++++	+++++
		−	− 300,000	±	±	±
Isoleucine	0.5	+	++++ 920M	+++++	+++++	+++++
		−	− 13,000	−	−	−
Leucine	0.5	+	++ 410M	+++++	+++++	+++++
		−	− 3,000	−	−	−
Methionine	0.5	+	++++ 730M	+++++	+++++	++++
		−	− 2.44M	±	++++	+++++
Tryptophane	0.5	+	++++ 580M	+++++	+++++	+++++
		−	− 43,000	−	−	−
Histidine	0.5	+	± 25.8M	+++++	+++++	+++++
		−	− 13,500	−	−	−
Lysine	0.5	+	++++ 820M	+++++	+++++	+++++
		−	− 27,000	−	−	−
Phenylalanine	0.5	+	− 463,000	+++	++++	++++
		−	− 280	−	−	−
Threonine	0.5	+	± 13.5M	++++	+++++	+++++
		−	− 1,630	−	−	−
Valine	0.5	+	++++ 920M	+++++	+++++	+++++
		−	− 72,000	−	−	−

strain "Coli" with the result that in several of these experiments a point was reached (at 1 per cent methionine) where growth was better

in the sulfanilamide cultures than in the controls. In other words, *sulfanilamide interfered with the bacteriostatic effect of methionine.*

TABLE IX

The Effect of Increasing Concentrations of Methionine upon its Antibacteriostatic Action in S1G1 Medium

Strain = "Coli"

METHIONINE CONCENTRATION	COLONY COUNTS—HOURS AFTER INCUBATION							
	24		48		72		120	
	Control	$PABS_{10}$	Control	$PABS_{10}$	Control	$PABS_{10}$	Control	$PABS_{10}$
mgm. %								
0	1420M	.06M	++++	−	1800M	.65M	+++++	−
	911M	.16M	1600M	.184M	1800M	1.40M	600M	45M
	820M	.08M	1300M	.490M	1900M	.80M	800M	12.5M
	680M	.002M	1240M	.003M	1900M	.004M	1300M	.057M
1.0	450M	.600M	+++++	−	1000M	590M	+++++	+++++
4.3	560M	.416M	660M	1.04M	1020M	1.50M	600M	1700M
8.6	730M	.560M	1040M	1.9M	810M	500M	700M	1700M
86]	810M	1.20M	1140M	6.8M	1850M	970M	1000M	1600M
200	1270M	.20M	++++	−	1118M	1590M	++++	++++
	830M	.90M	1800M	6.5M	2500M	870M	700M	1800M
	1000M	.27M	1500M	3.3M	2000M	81M	1000M	1200M
500	++++	−	++++	±	+++++	++++	++++	+++++
	930M	1.61M	1400M	6.3M	1800M	510M	990M	1090M
	990M	3.8M	600M	1110M	++++	++++	++++	++++
730	330M	.34M	++++	5.6M	++++	410M	++++	++++
860	4.9M	1.2M	280M	390M	280M	830M	60M	180M
1000	170M	.764M	+++	+	1200M	440M	++++	++++
	<100,000	1.7M	<1000	60M	18	1000M	2	40M
	4	.96M	2	44M	2	810M	2	120M
	12M	5.5M	100M	380M	++++	++++	++++	++++
	12M	5.5M	110M	340M	++++	++++	++++	++++
	37M	37M	60M	310M	++++	++++	++++	++++
	22M	29M	380M	260M	++++	++++	++++	++++
1500	<10	<10	−	−	−	−	−	−
1720	<100,000	<100,000	<2	10	<2	<2	<2	<2
2000	<100,000	<100,000	−	−	−	−	−	−

Studies upon Molecular Equivalents of Methionine and Sulfanilamide

In order to determine whether the antagonism between methionine and sulfanilamide is strictly dependent upon the relative concentrations of the two substances, they were studied in molecular proportions. Some of these proportions are shown in Table IX. (The

molecular equivalent of 10 mgm. per cent sulfanilamide is 8.6 mgm. per cent methionine.) Later, molar solutions of the two compounds in S1G1 were compared. The results of this study are shown in Table X. It is seen, in the first place, that very much less than 1 mgm. per cent methionine interferes with the bacteriostatic action of

TABLE X

Growth of E. coli in Various Proportions of Sulfanilamide and Methionine

METHIONINE CONCENTRATION		SULFANILAMIDE CONCENTRATION										
		Mgm. %										
Mgm. %	Molar	860	344	172	86	34.4	17.2	8.6	3.44	1.72	0.86	0
		Molar										
		1/20	1/50	1/100	1/200	1/500	1/1000	1/2000	1/5000	1/10,000	1/20,000	
1490	1/10	−	−	−	−	−	+	+	−	−	−	−
745	1/20			−	−	−	+	+				+
298	1/50			−		−						+
149	1/100				−		+	+				+
74.5	1/200				−		+	+				+
29.8	1/500					−	+	+				+
14.9	1/1000					−	+	+	+	+		+
7.45	1/2000						+	+	+	+		
2.98	1/5000						+	+	+			
1.49	1/10,000						+	+	+	+		
0.745	1/20,000						+	+	+	+		
0.298	1/50,000						+	+				
0.149	1/100,000						+	+				
0.0745	1/200,000						+	+				
0.0298	1/500,000						−	+				
0.0149	1/1,000,000						−	+				
0.00745	1/2,000,000						−	−				
0.00298	1/5,000,000						−	−				
0					−		−	−	+	+	+	+

+ = growth within 7 days; − = no growth within 7 days.

8.6 and even of 17.2 mgm. per cent sulfanilamide. There is, however, no constant ratio between the amount of sulfanilamide used and the amount of methionine required to neutralize it. Thus a millionth molar methionine interfered with the bacteriostatic action of 1/2000 molar sulfanilamide, but neither 1/500,000 molar nor 1/200,000 molar methionine had any effect on 1/1000 molar sulfanilamide and concen-

trations of sulfanilamide which exceeded 17.2 mgm. per cent were bacteriostatic for "Coli" in S1G1 no matter how much methionine was added. On the other hand, sulfanilamide concentrations of 3.44 mgm. per cent or less were not completely bacteriostatic; "Coli" grew out in them to more than 500 million in two to three days. There is therefore a very narrow range of sulfanilamide concentrations over which methionine can be shown to exert its effect. At the same time the range of effective concentrations of methionine is extremely wide—from 1/20 to 1/1,000,000 molar.

The Effect of Methionine Upon the Antibacterial Action of Sulfapyridine and Sulfathiazole on E. Coli

If the antagonistic action of methionine on sulfanilamide is related to the mode of action of the latter, one would expect methionine to interfere also with the antibacterial properties of sulfapyridine and sulfathiazole, for it is generally believed that all of the sulfonamide compounds act through the same mechanism. Methionine in 0.2 per cent, and 1 per cent concentrations had no visible effect on the bacteriostatic action of 10 mgm. per cent or even of 5 mgm. per cent sulfapyridine and sulfathiazole on the three strains of E. coli. This amino acid did, however, counteract the bacteriostatic action of 1 mgm. per cent of sulfapyridine on all three strains and of 0.5 mgm. per cent sulfathiazole on two of them. With strain "Coli" the antibacteriostatic action of methionine on sulfathiazole was not demonstrable until the drug concentration had been reduced to 0.1 mgm. per cent.

The Effect of Methionine on the Rate of Multiplication of E. Coli

It was recognized that the antagonistic action of methionine upon sulfanilamide might be due to an enhancing effect upon the growth of the micro-organisms. To test this possibility the rate of multiplication of strain "Coli" was studied in S1G1 with and without methionine and in the presence and absence of sulfanilamide. In the first experiments a 200 mgm. per cent concentration of methionine was used. Here it was found that the amino acid did have a slightly stimulating effect upon the rate of reproduction of "Coli". From the second until the twelfth hour after inoculation, there were from 2 to 6 times as

many micro-organisms in the cultures containing methionine as in those which did not. When smaller amounts of methionine (down to 0.015 mgm. per cent) were used, growth slightly better than in the control was again noted at the fourth, eighth, and twelfth hours after inoculation. But with large amounts of the amino acid—745 and 1,000 mgm. per cent—the inhibitory action of methionine itself came into play and there was poorer growth in its presence than in the control. At the same time, however, there was definite interference with

TABLE XI

The Rate of Multiplication of E. coli in the Presence of Various Concentrations of Methionine

METHIONINE CONC.	EXPERIMENT NUMBER	CONTROL						10 MGM. % SULFANILAMIDE						
		Number of bacteria per cc. at — hrs. after inoculation												
		0	4	8	12	24	48	0	4	8	12	24	48	72
mgm. %														
0	Av. of 7	162	761	32.4T	2.1M	976M		299	610	3733	10.6T	99T	1.1M	900T
0.015	1	76	760	41T	3.2M	620M		98	380	900	3400	640T	2.09M	3M
0.15	1	104	950	58T	5.6M	560M		98	800	900	4600	1.39M	3.64M	570M
1.0	2	284	2090	119T	28.3M	580M		202	1200	5500	26T	5.4M		
5.0	2	234	2580	196T	29.6M	680M		198	1550	11.9T	51T	4.24M		
20	2	184	3220	330T	18.8M	800M		218	1500	20.1T	86T	6.95M		
100	2	284	3260	134T	28.5M	1.13B		198	2410	5600	124T	5.4M		
200	Av. of 5	182	1640	78.5T	9.3M	1.13B								
500	3	145	1600	124T	14.4M	990M	600M	95	1600	24.5T	750T	3.8M	1.1B	
750	1	90	750	10T	<100T	330M		105	750	3300	9200	340T	5.58M	410M
1000	3	150	320	12.5T	136T	22M	380M	135	790	15.4T	400T	29M	260M	
1500	1	130	<20			<10		160	180	200	<100	<10		

the bacteriostatic action of sulfanilamide. In the presence of 1,500 mgm. per cent methionine, without sulfanilamide, the bacterial count dropped in four hours from 130 per cc. to less than 20, and in 24 hours, the culture was apparently sterile. Sulfanilamide delayed the bactericidal action of this concentration of methionine, for in the presence of 10 mgm. per cent of this drug, there were a few more bacteria per cc. at four and eight hours than at the start. By 24 hours, however, this culture also was sterile. The rate of multiplication of "Coli" in the presence of various concentrations of methionine is shown in Table XI.

DISCUSSION

When E. coli is cultivated in a synthetic medium containing glucose, asparagin, ammonium citrate and electrolytes its growth is readily inhibited by sulfanilamide. As a rule the tube containing the drug remains clear for five days and plate counts at this time have shown one million to ten million bacteria per cc. Occasionally there have been as few as 2000 per cc. and, in rare instances, there has been a 3 plus growth with about 500 million organisms per cc. on the fifth day of incubation. The substitution of other carbohydrates for dextrose does not alter the bacteriostatic action of sulfanilamide and the presence of certain complete proteins has little or no effect in this respect. Peptone, however, as was shown some years ago by Lockwood, completely abolishes bacteriostasis by sulfanilamide and this property is now seen to be shared by casein hydrolysates and by certain combinations of amino acids.

When the "essential" amino acids were tested separately, methionine, alone of the ten, consistently counteracted the effect of sulfanilamide. Arginine and lysine gave some evidence of possessing antibacteriostatic power but it was observed irregularly (in three out of five tests with arginine and two out of four with lysine), and it was of a much lower order than that exhibited by methionine. Certain combinations of amino acids in which methionine was lacking, also inhibited the action of sulfanilamide. Six such combinations were tested and four of them were antibacteriostatic in at least one of the two to four experiments. Arginine was present in three of these four combinations, but the fourth was composed of isoleucine, leucine and tryptophane. Arginine was also present in the other two combinations which lacked methionine and in which sulfanilamide was bacteriostatic. There were only two combinations in which sulfanilamide was effective in spite of the presence of methionine; in both of them the control growth was poor. In the remaining ten combinations which contained methionine bacteriostasis was negligible or transitory; indeed, in a majority of the trials growth was better in the presence of sulfanilamide than it was in the controls. In short, of the ten "essential" amino acids methionine is by far the most active antisulfanilamide agent.

The antisulfanilamide action of methionine is visible at 48 hours, at which time cultures containing this substance and 10 mgm. per cent sulfanilamide show a + to ++++ growth. Counts at 24 hours have shown that there are over a million bacteria per cc. present while in the sulfanilamide controls, containing no methionine, there are 1000 to 100,000 organisms per cc. Methionine is effective in low concentrations but its action is limited to a very narrow range of sulfanilamide concentrations. It is toxic to E. coli in concentrations above 500 mgm. per cent. In this range, instead of seeing enhanced bacteriostasis, because of the additive effects of two toxic agents, one often finds the reverse. Sulfanilamide appears to neutralize the antibacterial effect of methionine.

There are three possible explanations for the antibacteriostatic action of methionine. First, it might act by stimulating the growth of the micro-organism during the early stages of its development. Plate counts made at 24 hours showed no difference in the growth in cultures containing methionine and in those lacking it (that is, in the absence of sulfanilamide), but when the rate of multiplication, during the period of logarithmic increase, was studied it was observed that reproduction was indeed faster in the presence of methionine in concentrations of 500 mgm. per cent and under. In concentrations above 500 mgm. per cent, however, methionine does not stimulate the growth of E. coli; on the contrary, as has already been pointed out, it depresses it. Yet at these levels the amino acid continues to interfere with the action of the drug (and the drug with the antibacterial effect of methionine). For this reason we believe that the antibacteriostatic action of methionine is not attributable to a stimulation of growth.

The second possibility is that methionine interferes directly and specifically with the drug. In discussing the mode of action of mercurial salts, Fildes wrote (3) that it was "not improbable that the growth inhibiting effects of antiseptics in general might be due to a specific poisoning of some essential reaction exerted through an interference with the catalyst or the essential metabolite". He added that this poisoning might be occasioned either by the inhibitor combining with the essential metabolite and forming an inert product, or by the inhibitor competing with the metabolite for the enzyme involved. While our work was in progress, Woods' interesting report (4) of the

neutralizing effect of p-aminobenzoic acid on sulfanilamide was published. The action of p-aminobenzoic acid is explained by Woods, in conformity with Fildes' hypothesis, on the basis of competition for the same enzyme—the competition being the result of the structural similarity of the two compounds. Since sulfanilamide and methionine are not related chemically their mutual antagonism cannot be the result of competition for the same enzyme. Whether Fildes' alternative suggestion, *i.e.*, combination of the two substances, might afford an explanation of the effect of methionine on sulfanilamide is doubtful. It is difficult to conceive of how the two could combine. Moreover, the absence of a constant ratio between the amount of sulfanilamide present in a culture and the amount of methionine required to neutralize it suggests that they do not combine.

Finally, the possibility is to be considered that methionine, and perhaps arginine and lysine, are not of themselves antibacteriostatic but that they are the precursors of a substance which has antisulfanilamide activity. As to what that substance is, we have no information at the present time. Following the reasoning of the English workers (3, 4, 5, 6) we are inclined to believe that it must be an essential metabolite, with the production of which, *by the usual process*, sulfanilamide interferes. Methionine may permit the formation of this substance *via* some other reaction upon which sulfanilamide has no effect.

However this may be, the antibacteriostatic action of methionine provides one more link in the chain of evidence which points to the conclusion that sulfanilamide acts by interfering with the metabolic processes of bacteria.

SUMMARY

1. Methionine, in concentrations of 0.015 to 1000 mgm. per cent, interferes with the bacteriostatic action of 8.6 to 17.2 mgm. per cent sulfanilamide and of smaller amounts of sulfapyridine and sulfathiazole, upon E. coli in a synthetic medium.

2. This action is not shared by seven of the other "essential" amino acids, nor by homomethionine, cysteine, S-methyl cysteine, homocystine or choline. Arginine and lysine exhibit it irregularly and to a much lower degree than methionine.

3. The antisulfanilamide effect of methionine is not the result of its acting as a growth stimulant.

4. Concentrations of methionine of 1 per cent and over exhibit an antibacterial action upon E. coli. Sulfanilamide appears to neutralize this action to a certain extent.

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STUDIES ON THE CHRONIC TOXICITY OF SULFAGUANIDINE (SULFANILYLGUANIDINE)

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The preparation and chemical and physical properties of sulfaguanidine (sulfanilylguanidine) were described by Marshall, Bratton, White and Litchfield (1). This compound was found to have a considerable degree of solubility in water and to be poorly absorbed from the intestine. Because this gave rise to a new therapeutic idea, the fundamental principle of which involved the achievement of a high concentration of the drug in the intestine and a low concentration in the blood and tissues, these investigators conducted a more extended pharmacological and therapeutic study. On the basis of experiments on mice, rats and dogs they concluded that, if the drug be given by mouth, it is no more, and probably is less toxic than sulfapyridine and sulfathiazole. It was found that it was conjugated in the mouse, rabbit, and man, but not in the dog, and that the toxic effects noted in rabbits were owing to the deposition of acetylsulfaguanidine in the collecting tubules and pelvis of the kidneys. These workers also demonstrated that the number of coliform bacteria in the faeces can be reduced by its administration. At the meeting of the Southern Surgical Association in December 1940 Firor (2) reported the use of sulfaguanidine in the pre-operative preparation and the post-operative care of patients undergoing operations on the large bowel. He stated that the reduction in the number of bacteria in the alimentary canal was not striking in all cases, but was always sufficient to permit of operation without the usual risk of infection. The use of sulfaguanidine in the treatment of dysentery in children was reported by Marshall, Bratton, Edwards, and Walker (3) and by Lyon (4). The results seemed to be sufficiently encouraging to warrant further clinical trial.

Since in previous studies sulfaguanidine had not been administered

to dogs for more than one week, and since monkeys (which are known to conjugate the sulfonamides much as does man) had not been used, the present study was undertaken to determine the pharmacological effects and pathological changes resulting from administration of the drug to dogs and monkeys (Macaca mulatta) for a period of one month. The drug was also administered to rabbits.

TECHNIQUE

Rabbits: Two experiments were conducted with rabbits (New Zealand Red males) for a period of from 6 to 14 days. In the first case five animals were given daily 1 gram of sulfaguanidine per kilogram of initial body weight; in the second, the same number of animals was given daily one half this amount. The drug was administered as a 25 per cent suspension in water once a day by means of a stomach tube. Hemoglobin determinations and red and white blood cell and differential counts were made prior to the first dose and were repeated on the sixth day. Routine clinical laboratory examination of catheterized urine specimens was carried carried out at the same times. Examination was also made of urine obtained directly from the bladder post-mortem. The levels of sulfaguanidine in the blood were determined on the second and sixth days, in the morning before administration of the drug and four hours after its administration. The animals were maintained on a mixed rabbit chow consisting largely of grain, and had access to drinking water at all times. Their initial body weights ranged from 2250 grams to 2500 grams. Necropsies were performed on all animals.

Dogs: Five male mongrel dogs ranging from 12 to 24 kilograms in weight were given daily for 28 days 1 gram of sulfaguanidine per kilogram of body weight. The drug was administered by means of a stomach tube as a suspension (approximately 25 per cent) in water in two equally divided doses at 8 a.m. and 4 p.m. The weights of the animals were recorded three times weekly. Hemoglobin determinations and red and white blood cell and differential counts were made prior to the beginning of the experiment and at weekly intervals thereafter. The levels of sulfaguanidine in the blood were determined once weekly, in the morning before administration of the drug and four hours after its administration. Routine clinical laboratory examination of catheterized urine specimens was carried out once a week, and the non-protein nitrogen content of the blood was determined prior to the beginning of the experiment and on the 26th day. The van den Bergh reaction was carried out at the same times. The amount of drug excreted in the urine by each dog during a 24-hour period was determined on the eighth and fifteenth days. Contamination of the urine by fecal matter was avoided by rendering the stools hard. This was accomplished by feeding the dogs a low-residue diet consisting solely of forty grams of nearly fat-free shin beef per kilo. This also assured slower movement of the drug through the gastro-intestinal tract, thus securing longer contact with the epithelium of the intestine as well as with its contents. The

diet was supplemented by the daily administration of a multivitamin capsule. The animals were killed on the 29th day by opening the chest under pentobarbital sodium anesthesia. Necropsies were performed and bacterial counts were made on fecal material obtained from the distal colon.

Monkeys: Sulfaguanidine was administered to six male monkeys (Macaca mulatta) whose original body weights ranged from 2.9 to 3.35 kilograms. One-half of a gram per kilogram of body weight was administered daily as a 10 per cent suspension in water by means of a stomach tube in two equally divided doses at 8 a.m. and 4 p.m. for a period of thirty days. The blood levels of the drug were determined once weekly, in the morning before administration of the first dose and four hours following its administration. Routine clinical laboratory examination of uncatheterized urine specimens was carried out every fifth to seventh day. Hemoglobin determinations and red and white blood cell and differential counts were made at weekly intervals. A diet, uniform in character and quantity, was provided throughout the period of observation. It consisted of fresh vegetables and fruit, bread, and hard-boiled eggs. The weights of the animals were recorded twice each week. Bacterial counts were made on stools obtained from three of the monkeys before the experiment was begun and again on the tenth and 25th days of drug administration. All the animals were killed on the 31st day by opening the chest after etherization; and necropsies were performed.

RESULTS

Rabbits: In agreement with the findings of Marshall, Bratton, White, and Litchfield, sulfaguanidine was found to be extremely toxic for rabbits, due apparently to the facts that rather large amounts of the drug are absorbed and a very high percentage of it is converted into the acetylated form. In consequence the collecting tubules of the kidneys are obstructed by acetylsulfaguanidine. Subsequently there is a further rise of the level of the drug in the blood, particularly of the acetylated form, and a retention of nitrogenous waste products occurs. Table I demonstrates that although the blood levels of Group II on the second day are considerably lower than those of Group I (particularly four hours following administration of the drug), there is no longer an appreciable difference between the two groups by the sixth day. Between 50 per cent and 85 per cent of the drug was in the acetylated form in the blood. It is of interest to note that Rabbit #10, which failed to conjugate the drug to any extent, remained well, was gaining rapidly in weight, and showed no pathological lesions when killed at the end of three weeks. The long survival period of Rabbit #1 was adequately explained by finding at post-mortem that

approximately two-thirds of one kidney was entirely free of crystalline deposits.

Examination of urine specimens either obtained by catheterization on the fifth day or taken directly from the bladder after death revealed a great amount of sediment. This was seen microscopically to be made up of very characteristic crystals. In the urine of six of the rabbits these were in the form of parallelogrammatic sheets or plates, the great majority of which were rectangular, varying from narrow oblong structures to squares; they ranged as a rule from 5 micra to 30 micra in width and from 15 to 30 micra in length. The urine of Rabbit # 10 contained only the amorphous granular material (almost entirely phosphates) observed in the urine of all rabbits subsisting on a high-cereal diet. The sediment of the urine of the other three contained diverse crystalline forms, prominent among these being needles, occurring singly and in sheaves, and small morular structures, again occurring singly or in small groups. The latter were about 10 to 15 micra in diameter and, in some instances, appeared to be rosettes made up of minute needle-like structures. In addition, four of the ten rabbits had albuminuria, and microscopic hematuria; two of these had gross hematuria and one had microscopic pyuria.

The sediment from a pooled sample of urine was extracted with hot alcohol, using a reflux condenser. The alcoholic solution was then placed in the ice-box overnight. The following morning a crystalline material which had separated out was removed by filtration. By microscopic examination most of this was found to have the characteristic parallelogrammatic plate structure described above. On analysis the dried extract yielded 74.1 per cent acetylsulfaguanidine and 6.4 per cent free sulfaguanidine.

No significant changes were noted in the hemoglobin values or the red and white blood cell counts.

The marked loss in weight (Table I) was at least in part due to poor intake, which, in turn, was probably dependent upon uremia.

Necropsy findings

Except for small coccidial lesions of the liver, which were also present in four of the other rabbits, no pathologic findings were noted in Rabbit # 10. The remaining nine animals, however, showed characteristic and identical pathologic changes of the kidneys and spleen.

Grossly the kidneys were large, soft, rather boggy, and pale in appearance. The capsules stripped readily, revealing smooth external surfaces. On the cut surface the collecting tubules stood out as glistening yellowish streaks radiating fanwise from the central medullary papilla. This extraordinary delineation of the collecting tubules was found to be owing to the fact that they were packed with crystals, which could be scraped off onto the edge of the examining knife and in many instances were found to be free in the pelves, ureters, and bladder.[1] There was moderate pyelectasis in two animals and slight dilatation

TABLE I

Chronic Toxicity in Rabbits

GROUP	NUMBER	BLOOD CONCENTRATIONS IN MGM. PER CENT								BODY WEIGHT IN GRAMS			DAY OF DEATH
		Before 2nd dose		4 hours after 2nd dose		Before 6th dose		4 hours after 6th dose					
		Free	Total	Free	Total	Free	Total	Free	Total	1st day	6th day	14th day	
I†	1	0.95	4.7	11.3	20.8	2.0	8.1	8.6	16.7	2050	2000	1350	14
	2	1.05	4.1	10.4	25.7	28.6	94.5			2400			6
	3	1.0	4.7	9.4	18.9					2200	1950		6
	4	0.7	6.4	3.7	20.9	12.5	65.1			2450			6
	5	0.6	3.6	3.6	15.8	0.9	5.0	8.4	25.7	2150	1900		8
II‡	6	0.5	2.65	1.2	8.2	2.6	30.4	8.0	49.3	2400	2000		7
	7	0.6	3.1	1.8	15.2					2250			6
	8	0.5	3.7	1.9	12.7	4.0	49.8	22.8	83.0	2450	2150		8
	9	0.5	3.0	1.6	9.8	5.0	54.8	11.3	68.3	2300	1900		7
	10	2.2	3.0	10.2	12.0	3.9	5.4	10.8	11.5	2300	2350	2650	*

* Killed on 21st day.

† 1 gm. sulfaguanidine/kgm. daily.

‡ 0.5 gm. sulfaguanidine/kgm. daily.

of the proximal 3 or 4 centimeters of the ureters in one, though no obstructing masses were found. Microscopically the tubules and glomerular spaces were dilated, greatly so in many instances, and contained albuminous fluid, casts, and debris. The cells lining the collecting tubules were flattened and in three instances stained very darkly and were fragmented, suggesting that there had been a laying-down of calcium. The epithelium of the convoluted tubules was finely

[1] Material obtained by hot ethyl alcohol extraction of renal medullary tissue from two of the rabbits was shown by analysis of the dry extract to contain 25.7 per cent acetylsulfaguanidine and 2.3 per cent free sulfaguanidine.

granular and at times contained tiny vacuoles. In some areas the cells were so swollen that they projected into and obliterated the lumina; in others they were ruptured and displayed ragged borders.

On gross examination the spleen was seen to be much reduced in size. Excluding Rabbit #10, whose spleen weighed 1700 milligrams, not one of the spleens examined weighed more than 550 milligrams. The average weight was 470 milligrams, and the average length was slightly less than 3 centimeters. These measurements contrast with figures of 1812 milligrams and 5.5 centimeters obtained by averaging the weights and lengths of spleens taken from eight control rabbits of comparable body weight. Microscopically the changes responsible for this decrease in size were seen to be atrophy of the Malpighian corpuscles and splenic pulp. In all instances there was complete disappearance of the germinal centers of the Malpighian corpuscles and in some the marginal zone of lymphocytes had so markedly decreased in size that little remained but a few lymphocytes in a condensation of collapsed splenic stroma.

Nothing abnormal was noted in the liver grossly, except for small coccidial lesions. On microscopic examination, however, it was revealed that six of the rabbits were suffering from rather wide-spread granular degeneration of the hepatic cord cells. This degeneration was for the most part mid-zonal and para-central in distribution, though in one case it was distinctly and exclusively periportal.

Dogs: All the dogs remained well during the 28 day period of administration of the drug. There was no appreciable weight loss; three of the five dogs gained one-half kilogram or more during the experiment. No significant changes were noted in the hemoglobin values, the red and white blood cell counts, or the differentials. Before administration of the drug the average of the hemoglobin values for the five dogs was 13.02 grams, with extremes of 10.05 and 16.5; on the 26th day it was 12.4, with extremes of 10.75 and 15.0. The averages of the erythrocyte counts made at the same times were 7.25 million (5.9 to 9.7) and 6.9 million (5.61 to 8.7). Similarly the averages of the leucocyte counts were 12,370 (7,400 to 14,600) and 9,530 (6,950 to 12,100).

There were no constant urinary findings of note, except that on two occasions the sediment obtained by centrifugation of the urine of

Dog #438 was found to contain large numbers of white blood cells and needle-like crystals. Three of the remaining dogs passed needle-like crystals or irregularly fractured plate crystals on at least one occasion each. Prior to the experiment the average level of the non-protein nitrogen of the blood was 44 milligrams per cent with extremes of 42 and 50; on the 27th day it was 49 milligrams per cent with extremes of 45 and 53. The van den Bergh reaction was negative in each instance.

TABLE II

*Drug Levels in Dogs**

NUMBER		BLOOD CONCENTRATIONS IN MGM. PER CENT								
		1st day	2nd day		8th day		15th day		22nd day	
		4 hours after drug	Before drug	4 hours after drug	Before drug	4 hours after drug	Before drug	4 hours after drug	Before drug	4 hours after drug
434	Free	1.0	1.5	2.6	1.0	1.8	2.3	2.6	1.3	2.7
	Total	1.0	1.6	2.7	1.0	1.85				
435	Free	4.0	0.75	2.7	1.45	3.75	0.9	2.3	1.8	2.3
	Total	4.1	0.8	2.7	1.4	3.7				
436	Free	2.1	1.2	4.7	1.5	3.6	3.8	4.4	3.8	8.1
	Total	2.1	1.2	4.8	1.45	3.5				
437	Free	3.0	2.35	4.2	3.1	4.25	1.5	3.6	3.8	4.4
	Total	3.0	2.4	4.3	3.0	4.15				
438	Free	2.3	2.3	3.95	2.6	3.25	3.6	3.9	5.2	4.2
	Total	2.45	2.5	3.95	2.6	3.2				

* 1 gm. sulfaguanidine/kgm. daily in 2 equally divided doses.

In view of the large dosage being employed the levels of sulfaguanidine in the blood remained remarkably low throughout the experimental period (Table II). Since we soon found that dogs do not conjugate sulfaguanidine, just as they do not conjugate other sulfonamide compounds, we discontinued the practice of determining the total drug. Except for one isolated reading of 8.1 milligrams per cent the blood levels rarely exceeded 4. This is in agreement with previous workers. Analysis of 24-hour urine samples on all of the dogs on the eighth and fifteenth days revealed that there was an average ex-

cretion of 15.4 per cent of the total ingested drug on the first occasion' and 13.7 per cent on the second. The individual values ranged from 13.0 per cent to 17.2 per cent on the first, and from 11.6 per cent to 16.8 per cent on the second.

Necropsy findings

No pathologic findings of note were seen either grossly or microscopically except for bilateral renal lesions in Dog #437 and Dog #438. In each of these the normal clear-cut cortical striations ordinarily readily discerned on the cut surface of the kidney were for the most part obliterated by irregular greyish-white patches having their long axes parallel with the normal striations. Microscopically it could be seen that these were made up of collections of lymphocytes and monocytes occurring in areas where several glomeruli and their component tubule systems had been destroyed. In Dog #438 polymorphonuclear leucocytes were seen only occasionally, but in Dog #437 there were great collections of them constituting true abscesses. In each case a few areas in which renal tissue had been destroyed were replaced by well-formed scar tissue, indicating that the process was of considerable duration. An occasional hyaline glomerulus was observed. In a few areas the capsular spaces of the glomeruli contained albuminous fluid. No cultures were made but examination of microscopic sections with bacterial stains revealed no bacteria in the renal lesions.

Monkeys: All six of the monkeys remained well during the experimental period and showed no deviation from normal behavior and activity. There was no lagging of appetite. One animal lost 10 per cent in body weight, two gained respectively 6 per cent and 10 per cent, and three remained stationary. The hemoglobin concentrations and red and white blood cell counts remained normal. On the eighth day the average of the hemoglobin values for the six monkeys was 11.37 grams, with extremes of 10.1 and 13.9; on the 29th day it was 12.08, with extremes of 11.5 and 12.25. The averages of the erythrocyte counts made at the same times were 5.76 million (5.2 to 6.18) and 5.79 (5.48 to 6.08). Similarly the averages of the leucocyte counts were 14,600 (9,000 to 18,200) and 13,950 (9,500 to 19,600).

All the monkeys showed a trace of sugar in the urine at one time or another. This is a common occurrence in our monkeys because of the

high carbohydrate diet on which they subsist. Only one failed to show at one time or another characteristic parallelogrammatic plate crystals on microscopic examination of centrifuged urine specimens. The crystals were never abundant, and the urine specimens were always clear. The urine showed no other abnormalities. On the 26th day of the experiment the van den Bergh reaction was negative

TABLE III

*Drug Levels in Monkeys**

NUMBER		BLOOD CONCENTRATIONS IN MGM. PER CENT									
		2nd day		9th day		16th day		23rd day		30th day	
		Before drug	4 hours after drug	Before drug	4 hours after drug	Before drug	4 hours after drug	Before drug	4 hours after drug	Before drug	4 hours after drug
2	Free	0.9	1.7	0.9	2.4	0.7	1.6	1.0	0.55	0.8	0.85
	Total	1.4	2.6	1.65	3.7	1.15	2.6	1.7	1.25	1.2	1.9
3	Free	0.5	0.9	1.1	1.8	1.05	2.8	0.6	1.0	0.6	1.1
	Total	0.9	1.7	2.1	3.1	1.75	4.0	1.2	1.6	0.95	2.05
4	Free	0.9	2.2	1.15	2.75	1.3	2.1	1.45	2.2	0.8	1.45
	Total	1.25	3.9	1.6	4.3	2.15	3.45	3.1	3.75	1.6	2.7
6	Free	0.55	0.85	1.1	2.4	0.75	1.95	0.8	0.85	0.65	1.15
	Total	0.8	1.5	2.4	4.3	1.5	3.3	1.8	2.2	1.7	2.6
7	Free	1.0	0.95	0.9	1.4	0.75	1.3	0.5	0.7	0.5	0.8
	Total	1.3	1.5	1.65	2.8	1.4	2.3	0.9	1.25	0.85	1.4
9	Free	0.75	1.3	0.85	1.1	0.7	0.95	0.6	1.1	1.1	1.75
	Total	1.05	2.0	1.25	1.7	1.2	1.7	1.4	1.8	1.6	2.3

*0.5 gm. of sulfaguanidine/kgm. daily in 2 equally divided doses.

in each instance and there was no elevation of the non-protein nitrogen content of the blood, the average value being 43 milligrams per cent, with extremes of 37 and 50.

In view of what might be expected with comparable doses of other sulfonamides the blood levels of sulfaguanidine remained remarkably low throughout the experiment (Table III). Levels of more than 4 milligrams per cent of total drug were encountered on only two occasions, and comparatively few were more than 3. The percentage of acetyla-

tion varied but little from week to week. Thus, four hours after administration of the drug on the second day there was an average acetylation of 40 per cent, with extremes of 35 per cent to 47 per cent; on the sixteenth day, 39 per cent, with extremes of 30 per cent to 44 per cent; and on the thirtieth day, 45 per cent, with extremes of 24 per cent and 56 per cent.

Necropsy findings

The tissues of all of the monkeys were entirely normal with the two following exceptions. 1. Monkey #2 was found to have a small cavernous hemangioma of the liver. 2. A small semi-fluid caseous lesion, measuring about 1 centimeter in diameter, was found on the under surface of the right lobe of the liver of Monkey #7. This was suspected of being tuberculous, but a satisfactory block for microscopic study could not be obtained. There were also present many firm adhesions binding together the right lobe of the liver, the right kidney and adrenal, and the duodenum. Microscopically one small fresh tubercle was found in the lung of this monkey.

THE EFFECT OF SULFAGUANIDINE ON STOOL CULTURES

Bacterial counts of stools obtained from dogs and monkeys were determined by making agar plates of decimal dilutions of faeces in broth. The general morphology of the bacteria (whether bacilli or cocci) and their affinity for Gram's stain were ascertained by making smears of from 25 to 50 colonies in each instance. The results thus obtained indicate that sulfaguanidine has not in these animals effected a decrease in the number of bacteria. An interesting finding, however, has been a reversal of the differential count in all instances; that is to say, the Gram positive organisms, particularly the cocci, increase at the expense of the Gram negative bacilli. In some cases the Gram negative bacilli have apparently completely disappeared. Table IV clearly demonstrates this reversal of the differential count. Since no bacterial counts were made prior to the beginning of the experiment with the dogs, counts made on untreated dogs, sacrificed for other reasons, are presented for purposes of comparison.

In order to determine the rapidity with which change in the differential count occurs, bacterial studies were made at frequent intervals

with a monkey and a dog, neither of which were used in the studies on chronic toxicity (Table IV, Monkey #5 and Dog #422). It is evi-

TABLE IV

The Effect of Sulfaguanidine on the Bacterial Flora of Stools Obtained from Monkeys and Dogs†*

	NUMBER OF BACTERIA PER GRAM OF STOOL	DIFFERENTIAL COUNT IN PER CENT			REMARKS
		Gram neg. bacilli	Gram pos. cocci	Gram pos. bacilli	
Monkey #3	200 million	90	10	0	Prior to treatment
	4.4 billion	24	72	4	On 10th day of treatment
	800 million	42	36	22	On 25th day of treatment
Monkey #4	800 million	66	8	28	Prior to treatment
	3.6 billion	0	96	4	On 10th day of treatment
	1.4 billion	16	68	16	On 25th day of treatment
Monkey #6	700 million	50	18	32	Prior to treatment
	11.2 billion	0	84	16	On 10th day of treatment
	700 million	0	100	0	On 25th day of treatment
Monkey #5	50 million	66	4	30	Prior to treatment
	17 million (watery stool)	88	12	0	Prior to treatment
	100 million	10	52	38	On 2nd day of treatment
	160 million	16	22	62	On 4th day of treatment
	40 million	0	100	0	On 6th day of treatment
Dog #450	8 billion	100	0	0	Untreated
Dog #451	3.5 billion	60	40	0	Untreated
Dog #434	200 million	12	88	0	On 29th day of treatment
Dog #435	9 billion	8	92	0	On 29th day of treatment
Dog #436	2.4 billion	0	100	0	On 29th day of treatment
Dog #422	20 million	68	24	8	Prior to treatment
	45 million	92	8	0	Prior to treatment
	22 million	36	32	32	On 2nd day of treatment
	No count could be made	Plates overgrown with B. proteus			On 4th day of treatment
	11 million	16	76	8	On 6th day of treatment
	55 million	12	84	4	On 8th day of treatment

* 0.5 gm./kgm. daily in 2 equally divided doses.

† 1 gm./kgm. daily in 2 equally divided doses.

dent from these experiments that reversal of the differential begins almost immediately. In the case of Monkey #5 this was especially striking.

BLOOD CONCENTRATION-TIME CURVES

Because of the wide-spread interest aroused by the discovery of Marshall, Bratton, White, and Litchfield that sulfaguanidine is poorly absorbed from the intestine, it was deemed desirable to make studies of blood concentration-time curves in monkeys, employing several other sulfonamide compounds for purposes of comparison. The drugs were administered by stomach tube as 10 per cent suspensions in water,

TABLE V

*Blood Concentration-Time Curves**

		DRUG CONCENTRATIONS IN MGM. PER CENT									
		1 hour after drug		3 hours after drug		5 hours after drug		7 hours after drug		24 hours after drug	
		Free	Total	Free	Total	Free	Total	Free	Total	Free	Total
Monkey #96	Sulfaguanidine	0.75	0.8	2.0	2.1	2.0	2.4	1.4	2.1	0.75	0.8
	Sulfapyridine	1.2	1.95	1.9	4.25	4.05	7.85	2.1	6.3	0.1	0.85
	Sulfamethyl-thiazole	2.1	2.43	4.3	5.15	2.65	3.6	1.85	3.0	0.58	0.95
	Sulfathiazole	1.8	2.05	4.1	4.8	3.3	4.05	1.45	1.75	0.4	0.45
Monkey #8	Sulfaguanidine	0.8	0.95	1.05	1.5	0.8	1.5	1.0	1.7	0.7	0.95
	Sulfathiazole	2.3	3.0	4.55	6.1	2.15	3.1	2.3	3.2	1.0	1.6
	Sulfanilamide	5.1	5.9	26.4	29.7	35.1	38.0	29.7	35.3	3.2	6.0
Monkey #10	Sulfaguanidine	0.65	0.75	1.15	1.4	0.6	1.05	0.6	0.95	0.35	0.4
	Sulfathiazole	4.55	5.45	6.3	7.5	6.1	7.2	2.35	3.15	0.45	0.8
	Sulfanilamide	11.2	12.8	25.2	27.8	29.7	34.1	25.1	30.2	2.5	4.2

* 0.4 gm./kgm.

and a dosage of 0.4 grams per kilogram was employed for each drug. The curves have been converted into tabular form in order that the levels attained with the various drugs may be more readily compared (Table V). These experiments bear out the claims made for sulfaguanidine.

COMMENT

The toxicity of sulfaguanidine for rabbits is adequately explained on the basis of good intestinal absorption and excessive acetylation. The concentrations of the drug in the blood, and the urinary findings

(both microscopical and chemical) testify to this during life, as do the gross and microscopic appearance of the kidneys and chemical analysis of medullary tissue following death. The renal, splenic, and hepatic lesions were nearly identical with those described by Rake, van Dyke, and Corwin (5) as occurring in mice receiving sulfapyridine or sulfathiazole. Study of the kidneys of Dog #437 and Dog #438 suggests that crystals of the free drug, if present in sufficient quantity, may cause renal damage, but a bacterial origin for the abscesses was not excluded. Rake, van Dyke, and Corwin (5) have, however, described instances of "crystal pyelonephritis" occurring in monkeys as the result of sulfapyridine or sulfathiazole therapy and these resembled closely the lesion seen in the dogs.

The bacterial counts and differential studies indicate that sulfaguanidine is deleterious to the Gram negative organisms of the faeces. The failure of the drug to reduce the total number of organisms is owing to an increase in Gram positive cocci, which under normal circumstances may be kept in abeyance by the coliform bacteria.

The findings of these experiments seem to warrant the statement that under ordinary conditions any animal which absorbs sulfaguanidine poorly and does not acetylate it to excess can ingest the drug over a relatively long period of time, at least four weeks, without ill effects. Such an animal is the monkey.

SUMMARY

1. The pharmacological effects and pathological changes resulting from chronic ingestion of sulfaguanidine (sulfanilylguanidine) were studied in rabbits, dogs, and monkeys.

2. In rabbits the drug was found to be readily absorbed and excessively acetylated. The very high concentrations of the conjugated drug in the blood resulted in death in 80 per cent of the animals in eight days or less. Necropsy revealed renal, splenic, and hepatic lesions.

3. Though no toxic manifestations were noted in dogs receiving 1 gram of sulfaguanidine per kilogram of body weight daily for a period of one month, microscopic study of tissues revealed that in two of the five dogs there was moderate "pyelonephritis" possibly resulting from irritation by crystals of the free drug.

4. Neither signs of toxicity nor pathological findings were observed in six monkeys receiving one-half of a gram of sulfaguanidine per kilogram of body weight daily in two equally divided doses for a period of one month.

5. In accordance with the findings of Marshall, Bratton, White, and Litchfield in mice and dogs sulfaguanidine is poorly absorbed from the intestine in dogs and monkeys. The concentration of the drug in the blood remained remarkably low throughout the experiments. Blood concentration-time curves in monkeys gave consistently low results when compared to similar curves employing other commonly used sulfonamide compounds.

6. Bacterial counts of stools obtained from dogs and monkeys demonstrated that sulfaguanidine does not cause a decrease in the total number of organisms. However, a marked suppression of the Gram negative bacilli and a concomitant increase in the Gram positive cocci was noted in all instances.

I wish to acknowledge the valuable aid I received from Dr. Geoffrey Rake in carrying out the bacteriological studies.

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THE FATE OF TREPONEMA PALLIDUM INOCULATED SUBCUTANEOUSLY INTO IMMUNE RABBITS

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When a susceptible animal host is infected with syphilis, there is evolved an active immunity against the invading organisms which eventually becomes sufficiently powerful not only to heal all manifest lesions of the disease, but also to render the animal resistant to reinfection, especially with homologous strains of spirochetes.

This immune state evolves slowly. In the rabbit it does not become fully manifest until at least 90 days following infection (1). Once fully developed, however, it is not dissipated by treatment with spirochetocidal drugs (2, 3).

The interpretation of this persistence of immunity following treatment has been the subject of considerable debate. Neisser (4) maintains that it depends upon the survival of foci of spirochetes resistant to treatment, *i.e.*, that it is an infection immunity. On the other hand, Chesney (5) believes that intensive arsenical treatment may sterilize the host of spirochetes without destroying the immune reaction.

Whether or not the persistence of immunity after treatment is due to the survival of the original infection, it is clear that virulent spirochetes of homologous strains fail to produce visible lesions when inoculated into animals which have been treated in the late stages of the disease (*i.e.*, 90 or more days after the first infection). It is pertinent then to inquire into the ultimate fate of these spirochetes. Do they permeate the tissues of the host as a "symptomless reinfection," or are they destroyed by the immune mechanisms of the host; and if so, where and how?

In certain other infectious diseases, micro-organisms deposited in the tissues of the immune body are not only prevented from becoming

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generalized, but are known to be immobilized and destroyed at the site of inoculation (6, 7). As Rich (8) has pointed out, this local fixation and destruction of bacteria is not due to the mechanical effects of an accelerated inflammatory response but rather to specific local antibody reactions, such as agglutination and phagocytosis.

In syphilitic infections, the situation is less clear. Phagocytosis of spirochetes has never been demonstrated conclusively. Moreover, the body's defenses against this disease are relatively incompletely developed. Thus, in the rabbit if the second inoculation (with the homologous strain) is made intravenously or upon the surface of a granulating wound, the immune mechanisms may be so overwhelmed that reinfection may occur, although even in these instances no lesions develop (9). However, if reinoculation is made intratesticularly, intracutaneously, or subcutaneously, reinfection is almost always completely suppressed (10).

Chesney and Kemp's experiments (3) would seem to indicate that spirochetes inoculated intratesticularly into "immune" animals (*i.e.*, rabbits treated on the average of 192 days after infection, with arsphenamine, six injections at intervals of one week, each dose 10 mg. per Kg.) do not become generalized, since subsequent lymph node transfers were negative. Kolle and Prigge (11) however, have obtained positive lymph node transfers under somewhat similar experimental conditions and interpret their results as indicating the occurrence of "symptomless reinfections." These latter authors' treatment scheme (three injections of neoarsphenamine, each dose 0.1–0.15 gm. per Kg.) was less prolonged than was Chesney and Kemp's; and, since Worms (13) and Prigge (14) have shown that lymph nodes removed soon after the cessation of short courses of treatment may fail to be infectious whereas those removed considerably later may be, it is possible that Kolle and Prigge's positive transfers were due to treatment-resistant foci of the original infection.

There is some reason to believe that under certain experimental conditions, spirochetes inoculated into immune animals are not only prevented from disseminating throughout the body but are actually destroyed at the point of entry. This evidence is in the form of histologic studies by Strempel and Armuzzi (15) and Tani and Aikawa (16). Both groups implanted pieces of testis rich in virulent spiro-

chetes *subscrotally* in normal and immune animals, and at intervals thereafter excised the implant together with the surrounding tissues, studying the localization of the organisms with silver stains. Strempel and Armuzzi's rabbits were untreated and possessed an infection immunity. Tani and Aikawa used rabbits which had received neoarsphenamine administered late in the disease. Both concluded that whereas in normal animals spirochetes spread rapidly from the implant into the surrounding tissues; in immune animals, the organisms remained fixed *in situ*, gradually disappearing, first from the periphery of the implant and later from the center, as though by lysis. Strempel and Armuzzi's implants became spirochete-free histologically within 27 days; Tani and Aikawa's within 21 days.

Histologic studies of this sort are open to several objections. Staining methods for the demonstration of spirochetes in tissue sections are notoriously capricious. Moreover, the possibility of occlusion of the lymphatic channels resulting from the preceding orchitis cannot be dismissed, for, as Menkin (17) has emphasized, this may be a factor in preventing spread of organisms. Then too, the migration of some of the spirochetes to the neighboring lymph nodes is a possibility which cannot be excluded. Lastly, histologic studies afford no indication of the viability of the organisms.

It seemed advisable therefore to approach the problem by selecting a distant site for reinoculation, and by removing the implant and adjacent lymph nodes for inoculation into normal animals in order to determine (a) how long the reinoculated organisms in the implant remain viable, and (b) whether or not they make their way to the adjacent lymph node. The selection of a distant site for reinoculation (thigh) rules out the effect of any obliterative lymphangitis that may have resulted from the original infection.

EXPERIMENTAL

Two groups of rabbits were used: (I) normal animals and (II) animals which had been treated intensively with arsphenamine late in the course of syphilitic infection, and which on the basis of previous experience were considered immune although sterilized of spirochetes. The Nichols strain of *T. pallidum* was used for all inoculations. Treatment of group II animals was begun 174–178

days after infection by intratesticular inoculation, at a time when all manifest lesions had subsided. Six injections of arsphenamine were

TABLE I

Results of Subcutaneous Inoculations in Normal Rabbits

TIME AFTER IMPLANTATION	RABBIT	IMPLANT			LYMPH NODE	
		Darkfield		Result	Transfer rabbit	Result
		Spirochetes	Motility			
days						
2	6360	0–1/field	Sluggish	Positive	6335	Positive
					6336	Positive
	6361	2–3/field	Sluggish	Positive	6334	Positive
					6384	Inconclusive*
4	6362	0–2/field	Sluggish	Positive	6344	Positive
					6348	Inconclusive*
	6363	0–2/field	Sluggish	Positive	6375	Positive
					6376	Positive
8	6364	0–2/field	Sluggish	Positive	6351	Positive
					6392	Positive
	6365	0–1/field	Sluggish	Positive	6356	Positive
					6357	Positive
14	6366	2–3/field	Sluggish	Positive	6401	Inconclusive*
					6402	Negative
	6367	2–3/field	Sluggish	Positive	6358	Positive
					6399	Positive
Normal controls (implant not removed)	6368	No visible lesion at site of implantation. Metastatic lesion of right epididymis and testis				
	6369	No visible lesion at site of implantation. Metastatic periostitis of left radius				
Virulence controls	6370	Intratesticular inoculation. Result inconclusive.*				
	6371	Intratesticular inoculation. Typical primary orchitis at 16 days				

* Rabbit died.

given intravenously at weekly intervals, each individual dose being 20 mg. per Kg. of body weight.

Test inoculations were made by the subcutaneous implantation of a

TABLE II

Results of Subcutaneous Inoculations in Immune Rabbits

TIME AFTER IMPLANTATION	RABBIT	DAYS SINCE LAST TREATMENT	IMPLANT				LYMPH NODE	
			Darkfield		Transfer rabbit	Result	Transfer rabbit	Result
			Spirochetes	Motility				
days								
2	6092	54	0–1/field	Sluggish	6373	Positive	6408	Negative
					6403	Positive	6409	Inconclusive*
	6088	32	0–1/field	Active	6410	Positive	6412	Inconclusive*
					6411	Positive	6413	Negative
4	6093	54	0–1/field	Very sluggish	6414	Negative	6418	Negative
					6415	Negative	6419	Negative
	6119	32	0–1/field	Very sluggish	6416	Inconclusive*	6417	Negative
					6395	Inconclusive*	6383	Negative
8	6094	54	0		6427	Negative	6429	Negative
					6428	Negative	6430	Negative
	6130	32	0		6420	Negative	6422	Negative
					6421	Inconclusive*	6423	Negative
14	6096	54	0		6434	Negative	6432	Negative
					6435	Negative	6433	Negative
	6131	32	0		6436	Negative	6439	Inconclusive*
					6437	Negative	6440	Negative
21	6103	54	Absorbed			Negative	6443	Negative
						Negative	6444	Negative
	6134	32	0		6447	Negative	6445	Negative
					6448	Negative	6446	Negative
Immune controls (implant not removed)	6106	54	Implant absorbed at 21 days. Blood flocculation test negative. No metastatic lesions					
	6137	32	Implant absorbed at 21 days. Blood flocculation test negative. No metastatic lesions					
Virulence controls	6372	Normal	Intratesticular inoculation. Typical primary lesion at 30 days. Metastatic lesion in tail					
	6374	Normal	Intratesticular inoculation. Typical primary lesion at 14 days					

* Rabbit died.

piece of testis about 1.5 cms. in diameter (removed at the height of acute orchitis, and demonstrated to contain many actively motile *T. pallida*) into the inner aspect of the left thigh. These inoculations were made either 54 or 32 days after the last dose of arsphenamine, an interval sufficient to allow the effect of antisyphilitic treatment to wear off. At intervals thereafter (2, 4, 8, 14 and 21 days), the implant was removed, as was the left inguinal lymph node. Each was macerated separately and suspended in physiologic salt solution. The implant suspensions were examined by darkfield illumination, the lymph node suspensions were not. Each suspension was then divided into two equal parts and inoculated intratesticularly into each of two normal rabbits. When motile *T. pallida* were demonstrated in implants removed from normal animals (group I), no transfers were made, it being assumed that the organisms were pathogenic. Implant transfers were made from all immune animals (group II) regardless of the darkfield findings.

Two sets of controls were run. In two animals of each of groups I and II the implant was left *in situ*. The virulence of the spirochetes used was checked further by inoculating two normal rabbits intratesticularly with the same material as was used for the subcutaneous implantations.

The results of this experiment are summarized in tabular form. In Table I are represented the findings in the group of normal rabbits; in Table II, the results with the immune animals.

DISCUSSION

Under the conditions of this experiment, the differences between normal and immune animals in their reaction to reinoculation are quite apparent. In the normal rabbits, spirochetes readily permeated the regional lymphatics, and remained viable in the implant, as judged by darkfield examination, as long as the implant was studied (14 days). In the immune rabbits, no pathogenic spirochetes were ever demonstrated in the inguinal lymph nodes by the transfer method. Organisms could be demonstrated in the implants by darkfield examination up to 4 days after implantation, but only up to 2 days by transfer to normal animals.

In contrast to what occurs when implantation of infected tissue

is made subscrotally, no visible lesion developed on the thigh at the site of implantation in any of the four control animals. In the immune rabbits this is readily explained, for the spirochetes apparently were immobilized and destroyed. In the normal animals the mechanism responsible for the suppression of the chancre is not clear. Microscopic study of the site of implantation was not made. That these non-immune control animals were infected is clear, since metastatic lesions developed.

On the basis of these experimental results, it seems probable that in the immune rabbit, subcutaneously-inoculated *T. pallida* of homologous strain do not permeate the lymphatics, but are localized at the site of inoculation and subsequently destroyed by the immune mechanisms of the host. The actual immobilization and destruction of spirochetes is probably accomplished by a local antigen-antibody reaction, as immune antibodies are gradually brought into contact with the invading organisms.

CONCLUSION

Homologous strains of *T. pallida* inoculated into the subcutaneous tissues of the inner aspect of the thigh of immune rabbits do not migrate to the regional lymph nodes but are immobilized and destroyed *in situ* by the immune processes of the host.

The author wishes to express his appreciation of the advice and assistance of Dr. Alan M. Chesney, under whose supervision this experiment was carried out.

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CONGENITAL ABSENCE OF THE SEPTUM PELLUCIDUM

A Case Diagnosed Encephalographically and Associated with Congenital Amaurosis

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According to Dolgopol (1), only four cases of absence of the septum pellucidum in fully developed brains, and two in fetal brains have been reported. The first of these was that of Tenchini (2) in 1880, and the second that of Hochstetter (3) in 1925. Prior to this, Hochstetter had observed absence of the septum pellucidum in otherwise well-formed brains of fetuses about the third and fifth month of gestation. He believed congenital absence of the septum pellucidum was not rare and usually escaped attention. In 1930 Hahn and Kuhlenbeck (4) also discovered the anomaly in the dissecting room, but no information on the history of the patient was obtained. Dolgopol added an additional case discovered at autopsy in 1938.

In 1935 Dyke and Davidoff (5) demonstrated this anomaly encephalographically for the first time in a 23 year old woman with postencephalitic disorders of behavior. Undoubtedly Dandy's (6) description of the pneumographic picture of congenital cerebral cysts of the cavum septi pellucidi and cavum vergae in 1931, and those by Davidoff and Dyke (7) and Hyndman and Penfield (8) of absence of the corpus callosum led to a more easily appreciated picture of absence of the septum pellucidum. Sfintzescu and Mihailescu (9) reported a case found ventriculographically in 1936, and although others may have been overlooked, those diagnosed pneumoencephalographically have at all events been extremely uncommon, and for that reason as well as for the fact that the present case is the youngest so diagnosed, it was believed worthy of publication.

REPORT OF CASE

History: R. W. (41-603), a 7 months old male infant, was admitted to the Children's Hospital of Los Angeles, February 14, 1941, and the following history obtained. Birth was normal and spontaneous at full term after a labor of 8 hours. Birth weight was 6 pounds and 11 ounces. There was no trouble with cyanosis or resuscitation and the feedings were taken well. A strabismus was noticed at 5 months, and about this time it was discovered that the infant was unable to see

FIG. 1. PHOTOGRAPH OF THE PATIENT

objects. At the age of 6 months he was able to sit up and at 6½ months stood in his crib with support. There had been no history of infectious disease and no convulsive seizures of any kind.

Examination: Revealed a well-developed and nourished baby of 7 months, able to sit up alone, but unable to notice objects brought before him (Fig. 1). The contours of the head were not unusual, the percussion note over the calvarium was not elevated, and there was no stiffness of the neck. The anterior fontanelle admitted two finger-tips, but was not under increased pressure. Head circumference

was 44.5 cm. The ear drums and nasal septum were intact, and the oral cavity was not remarkable. Chest expansion was equal and sufficient, the percussion note and breath sounds normal, and no râles were heard. Nothing unusual was discovered in the abdominal examination, and the extremities appeared to be

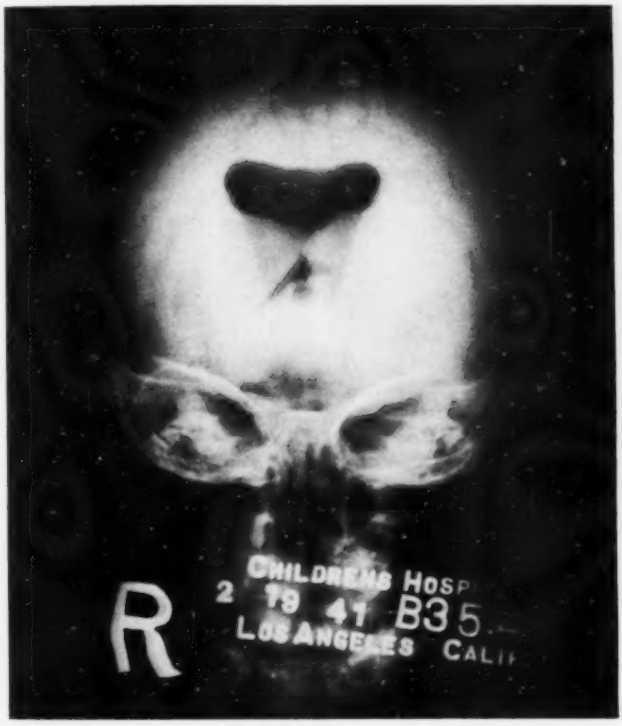

FIG. 2. ENCEPHALOGRAM. ANTEROPOSTERIOR VIEW SHOWING MEDIAL CONFLUENCE OF THE LATERAL VENTRICLES ANTERIORLY RESULTING FROM ABSENCE OF THE SEPTUM PELLUCIDUM

normal. In the neurological examination, the optic discs were seen only with difficulty and appeared to be pale. Subsequent examination by Dr. Rodman Irvine under ether anesthesia led him to the diagnosis of bilateral primary optic atrophy of undetermined origin, probably, however, on the basis of a congenital aplasia. The pupils failed to react to light and there was no blinking of the eyes when objects were brought suddenly toward them. There was a strabismus and

coarse nystagmus probably on the basis of the amaurosis. The corneal reflexes were present and equal, and there was no indication of facial paralysis. Hearing was present to the extent that the baby was startled when noises were made near

FIG. 3. ENCEPHALOGRAM. POSTEROANTERIOR VIEW REVEALING THE ANTERIOR CONFLUENCE OF THE LATERAL VENTRICLES AND THEIR NORMAL POSTERIOR SEPARATION

him. The uvula elevated in the midline, and there was no weakness of the shoulder or neck movements, and no tongue deviation was observed.

No paresis of the extremities, nor any trouble with coordination was evident.

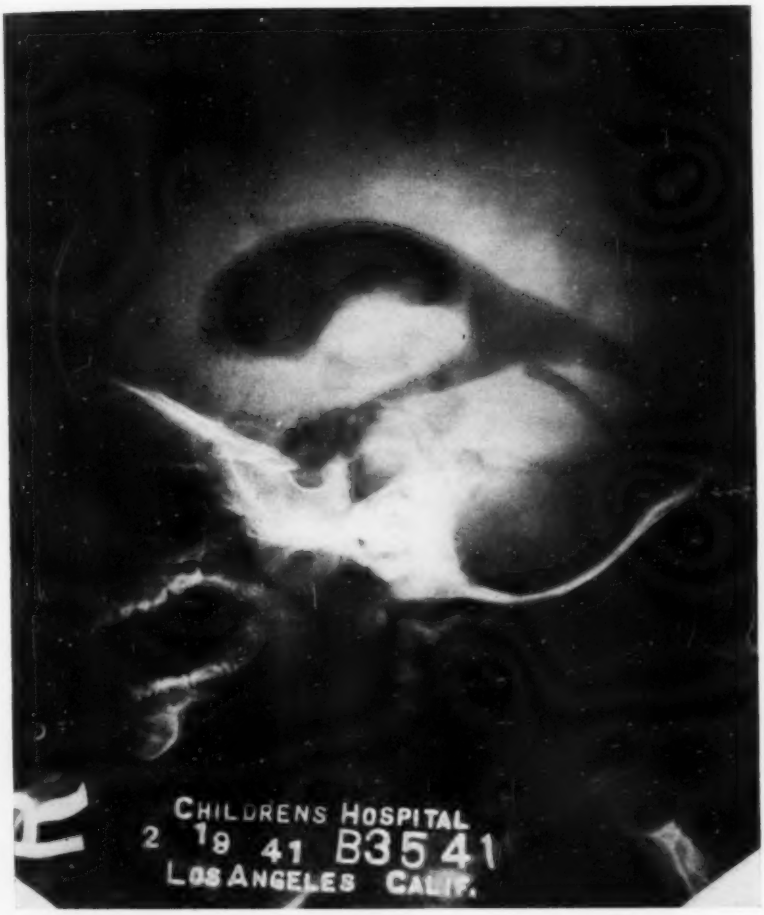

FIG. 4. ENCEPHALOGRAM. RIGHT LATERAL STEREOSCOPIC VIEW SHOWING THE COMMA-SHAPED SHADOW PRODUCED BY THE CONFLUENCE OF THE TWO LATERAL VENTRICLES DUE TO THE ABSENCE OF THE SEPTUM PELLUCIDUM

The extremities were withdrawn to painful stimulation. The deep reflexes were present and equal and no spasticity was noted.

Laboratory Data: Urinalysis revealed no albumin, sugar, or acetone, and micro-

scopically no casts or erythrocytes. Examination of the blood disclosed a hemoglobin of 58 per cent, an erythrocyte count of 4,450,000, and a leucocyte count of 8,850. Sixty-one per cent lymphocytes, 33 per cent polymorphonuclears, 1 per cent eosinophiles, and 5 per cent monocytes were recorded in the differential exami-

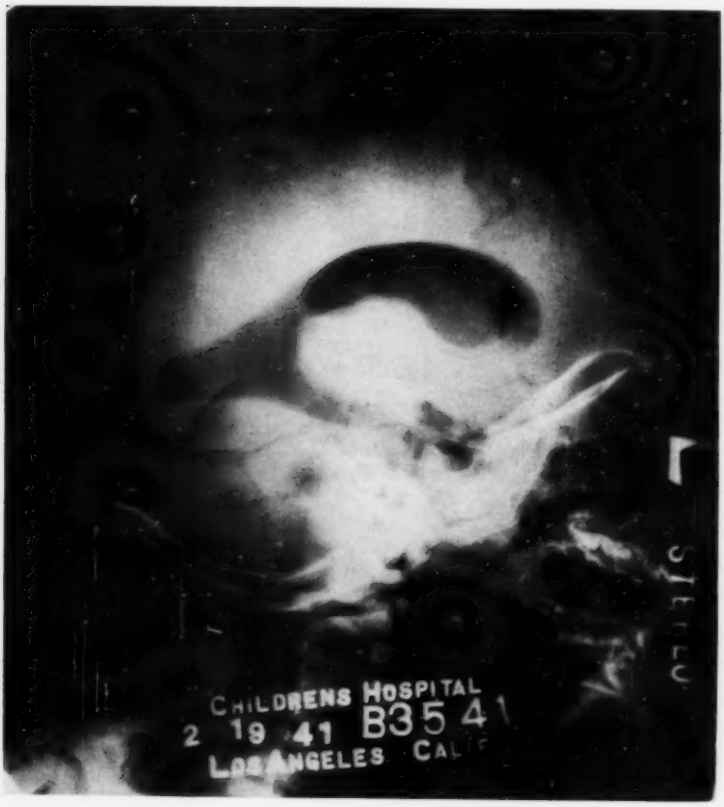

FIG. 5. ENCEPHALOGRAM. LEFT LATERAL STEREOSCOPIC VIEW DISCLOSING A SIMILAR PICTURE

nation. Both blood and spinal fluid Wassermann tests were negative. The spinal fluid protein determination was 33.6 mg. per cent. Roentgenograms of the skull and chest revealed nothing unusual.

Electro-encephalography, February 18, 1941, was reported by Dr. L. E. Travis

as showing long trains of relatively sinusoidal waves having a frequency of 3 to 5 per second, appearing mainly from the temporal and frontal regions; and irregular waves having a frequency of 1 to 3 per second appearing mainly from the occipital region.

FIG. 6. ENCEPHALOGRAM. LATERAL VIEW WITH THE OCCIPUT UP SHOWING THE SHADOW OF THE POSTERIOR HORN, PLUS THE DARKER SUPERIMPOSED SHADOW OF THE CONFLUENT LATERAL VENTRICLES

Pneumoencephalography was performed February 19, 1941 under sodium amytal and ether anesthesia. The initial spinal fluid pressure was slightly elevated. Thirty-five cc. of air was injected and a similar amount of spinal fluid withdrawn. This was done in exchanges of 5 cc. Roentgenograms revealed the unusual and

striking picture of congenital absence of the septum pellucidum. As has been so well illustrated by Dyke and Davidoff (5), in the anteroposterior and posteroanterior views the two anterior lateral ventricles appeared confluent medially with a normal posterior separation (Figs. 2 and 3). The usual linear vertical shadow produced by the septum pellucidum was therefore missing and at the midline anteriorly there was a nipple-like projection from the ventricles. The third ventricle was in the midline and appeared normal in size and shape. In the right and left lateral stereoscopic views, as well as in the lateral films with the brow and occiput up, the third ventricle, aqueduct and fourth ventricle appeared normal, and the comma-shaped shadow representing the confluent portions of both lateral ventricles at the midline was seen superimposed upon those of the bodies and the posterior half of the anterior horns of the lateral ventricles (Figs. 4, 5, and 6). It was believed the films were characteristic of no other anomaly than that of absence of the septum pellucidum. The infant tolerated the procedure well and was discharged February 22, 1941.

DISCUSSION

In the anteroposterior and posteroanterior views of the normal encephalogram, the septum pellucidum forms a well-demarcated vertical linear shadow, 2 to 3 mm. wide, between the air shadows of the two anterior lateral ventricles. In the absence of the septum pellucidum this shadow is missing, and the lateral ventricles are confluent anteriorly but separated normally posteriorly. In addition a small, nipple-like projection from the medial ventral portion of each lateral ventricle can be seen at the region of the dorsal surface of the anterior pillars of the fornix. Although in the lateral views in cases with a normal septum pellucidum, there may be an intensification of the air shadows of the two lateral ventricles where these are superimposed, in the absence of the septum pellucidum, in addition to this intensification, there is a third darker shadow, which represents the area where the ventricles are confluent.

While in the lateral films a communicating cavity of the septum pellucidum may reveal a picture quite similar to that of absent septum pellucidum, the diagnosis is clearly established in the anteroposterior and posteroanterior views by the presence of this air-filled space between the two lateral ventricles. If this cyst of the septum pellucidum does not fill with air, the lateral ventricles are then seen abnormally separated. Similarly, there can be no confusion with the striking "bat-wing" or bicornuate appearance of the ventricles in agenesis of the corpus callosum (10).

Dyke and Davidoff (5) have pointed out that in cases of internal hydrocephalus due to obstruction of the foramen of Monro by tumors of the third ventricle, secondary perforations of the septum pellucidum of varying size were seen at operation. With the exception of the hydrocephalus, they found the preoperative ventriculograms strikingly similar to those of absence of the septum pellucidum. There was, however, no third ventricle filling, the hydrocephalus was quite marked, and the anterior confluence of the lateral ventricles was less striking, so that in spite of the similarity, the picture was sufficiently different to be diagnostic. In numerous hydrocephalic films seen in children, the ventriculograms seldom reveal a picture which could be confused with absence of the septum pellucidum. It, therefore, hardly seems likely hydrocephalus alone can be responsible for the condition.

Thompson (11), who reviewed the abnormal conditions of the septum pellucidum, expressed the belief that certain cases of perforation of the septum pellucidum were almost without doubt congenital in origin, most of them exhibiting a cavum septi pellucidi communicating with the third and lateral ventricles without pathological lesions such as hydrocephalus.

Inasmuch as the development of the corpus callosum, the hippocampal commissure, and the septum pellucidum progress concomitantly, reaching completion by the fifth month, arrested development ordinarily can be expected to affect all of the structures and the extent of the anomaly will depend upon the time at which this arrest occurs (8). Towards the stage of completion of development, however, it does not seem unreasonable to expect that one of these structures may be more affected than another. In such manner an absence of the septum pellucidum may be predicated without obvious impairment of the others being evident encephalographically.

As a result of his investigations on the formation of the septum pellucidum and its cavum, Hochstetter (12) differed from the embryological point of view that the cavum of the septum pellucidum formed part of the median fissure, and was separated by adhesion of the median surfaces of the hemispheres along a line of closure reenforced by the rostrum of the corpus callosum. He observed that the cavum was formed within the thickened upper part of the lamina terminalis between the corpus callosum and the anterior commissure by means of resorptive processes. It was his belief that the process of resorption

might occasionally involve the walls of the cavity to a greater extent than normal with resultant disappearance of the walls of the septum pellucidum.

Although patients with agenesis of the corpus callosum may show no indication of mental retardation, epilepsy and impaired mentality have been the most frequent associated clinical manifestations of the condition. Moreover, the reported cases of cysts of the septum pellucidum have been similar in this respect. The patients in the five cases reported by Verga (6) in which the cavity was discovered at necropsy, were from psychopathic wards and revealed varying degrees of mental disturbance. Likewise those reported by Gibson (13) and Thompson (11) were psychopathic; in addition Thompson's patient was judged to be an imbecile. In one of Dandy's (6) patients, a boy of 4½ years, there was a history of retarded development and peculiar epileptic attacks of varying character.

It therefore seems reasonable to anticipate such peculiarities in subsequently reported cases of patients with absence of the septum pellucidum. It is not surprising that congenital amaurosis occurred in the presently reported case, for this is a not uncommon congenital abnormality (14).

Hochstetter's belief that congenital absence of the septum pellucidum is not rare and usually escapes attention may be borne out in later reports when the encephalographic features reported by Dyke and Davidoff become more appreciated.

SUMMARY

1. Encephalography in an infant of 7 months with congenital amaurosis revealed the characteristic features of congenital absence of the septum pellucidum.

2. The present case, the third reported since the first encephalographic description of the unusual anomaly in 1935 by Dyke and Davidoff, was so diagnosed at the earliest recorded age.

3. The medial confluence of the anterior lateral ventricles with normal posterior separation represent the main diagnostic features of the anteroposterior and posteroanterior views.

4. The differences between the encephalographic findings in agenesis of the corpus callosum and cysts of the septum pellucidum is discussed.

5. It is believed that as the encephalographic picture of the condition becomes appreciated, more of these cases will be described in the future.

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BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal.)

Synopsis of the Principles of Surgery. By JACOB K. BERMAN. 615 pp. Illus. $5.00. *C. V. Mosby Company, St. Louis, 1940.*

This book is exactly what the author intends it to be—a synopsis of surgical knowledge. It is a surprisingly compact and informative volume. There is no question that a handbook of this character fills a real need, both for the medical student and the busy practitioner. Dr. Berman has gone to great effort to make the subject matter accurate and concise. It is in no wise to be looked upon as a textbook but simply as a carefully arranged synopsis of surgery.

M. F.

The Medical Reports of John Y. Bassett, M.D., the Alabama Student. With an introduction by DANIEL C. ELKIN. 62 pp. Illus. $1.50. *Charles C. Thomas, Springfield, Ill., 1941.*

"The Alabama Student" is founded on one of Osler's happiest discoveries in the field of biography. The charming vignette is constructed from a series of letters written by Dr. John Y. Bassett to his wife, after he had left her, with many regrets, for a year of study in Europe; and from three articles which Bassett had published in Fenner's *Southern Medical Reports* appearing in 1849 and 1850.

These three articles form the basis of the brochure edited by Dr. Daniel C. Elkin. They are preceded by a foreword prepared by Dr. Elkin and are followed by an appendix consisting of a letter to Dr. Osler from Dr. Martin of Mobile which throws light upon the character and life of Bassett. Martin had known Bassett in Huntsville and admired him. Stimulated by Osler's essay on "The Alabama Student," he adds some picturesque details concerning the association of Bassett with Fenner. This little pamphlet, edited by Dr. Elkin, is therefore complementary to Osler's well known essay, and thus, in order to appreciate either of them, one must read both.

The first article is entitled "Report on the Topography, Climate and Diseases of Madison County, Alabama"; the second is noted as "Clinical Reports," and the third "Report on the Climate and Diseases of Huntsville and its Vicinity for the Year 1850." In these three brief articles Bassett gives us glimpses of a life so different from our own and of a form of medical practice so far removed from the present that it scarcely seems possible that he could have lived less than a hundred years ago. In sharp contrast to this background as a setting, his philosophy, his point of view, his approach to the insoluble medical difficulties with which he was

confronted is of our own times. This serves to transport us into his unbelievably crude surroundings and to reproduce for us the tragic and pathetic medical situations in which he found himself.

Though Bassett can be sharp in his criticism yet at times he is lightly discursive and often humorous. He touches on many subjects and the short articles, now so rare that they are almost unobtainable in the original, make good reading and are well worthy to be preserved.

W. T. L.

Science and Seizures. By WILLIAM G. LENNOX. 258 pp. Illus. $2.00. *Harper & Brothers, New York and London, 1941.*

Science and Seizures is written by Dr. William Lennox of Boston as a part of the educational program of the *Laymen's League Against Epilepsy.* Although it is intended to meet the needs of patients and of the general public for a better understanding of epilepsy and migraine, it has much to recommend it to physicians as well.

The larger part of the book deals with the practical problems of epilepsy which face the patient, his family and his physician. Besides explaining the nature of the disease in a simple and wholesome way, the book encourages cooperation with physicians toward improved care. From his wide experience Dr. Lennox is able to offer helpful suggestions to enable the patient to deal with his handicap and still live as normal a life as possible. For the family, the account of the disease should assist in discarding the traditional superstitition and disgrace surrounding the individual with seizures, and in realizing that the outlook is often far from hopeless.

Dr. Lennox discusses at some length the significance of the electroencephalographic findings in the disease. To some, it may seem that he places undue emphasis on the specificity of the findings in epilepsy and overstates the hereditary aspect of the disease as indicated by brain wave studies. Nevertheless, the discussion is so worded as to allay anxiety, and at the same time to unite those interested toward an active program of research.

A smaller section of the book deals in much the same way with migraine, a disorder closely allied to epilepsy.

Since *Science and Seizures* is being widely advertised to the public, physicians who handle patients with either migraine or epilepsy may find it advantageous to become acquainted with the book and its viewpoint.

E. M. B.

English-German and German-English Medical Dictionary. 1. Part. English-German. Fünfte, durchgesehene Auflage von DR. ADALBERT SPRINGER. 201 pp. R.M. 6. Franz Deuticke, Wien, 1939.

The third edition of this small dictionary added the terms coined in the period from 1891 to 1925. The fifth edition which is a reprint of the fourth (1933), includes the new terminology of the years since 1925. On the other hand a number

of words, especially those Latin expressions which are used identically in both English and German, are omitted so as to retain the original size of the book. The dictionary provides material which is rarely found in the average English-German lexicon; thus it is of great help for the German reader of English medical literature.

L. E.

Clinical Pellagra. By SEALE HARRIS, M.D., assisted by SEALE HARRIS, JR., M.D. 494 pp. Illus. $7.00. *The C. V. Mosby Co., St. Louis, 1941.*

The appearance of a new volume on clinical pellagra is most timely in view of the recent important developments in our understanding of this interesting disease. The first half of the book contains an exhaustive review of the early work on pellagra. Lengthy quotations from original sources should prove valuable to those concerned with the historical background, and the various etiologic theories which were propounded. Dr. Harris pays just tribute to such pioneer workers as Lombroso in Italy, Searcy, Chittenden, Goldberger and Wheeler in this country. The observation that canine blacktongue bears a close resemblance to human pellagra furnished the latter investigators with a useful method of studying the various manifestations of the disease. The discovery that experimental blacktongue could be produced at will by feeding dogs diets deficient in the vitamin B complex supported the theory that human pellagra might be a deficiency disease rather than an infection or toxic disorder as was originally supposed.

In 1937 Elvehjem reported that nicotinic acid, a simple chemical compound, was specific in alleviating the symptoms of canine blacktongue. Shortly thereafter it was conclusively demonstrated that nicotinic acid would relieve the majority of the symptoms of human pellagra. Dr. Harris is not satisfied with the theory that pellagra is due solely to vitamin deficiency. He believes with Sydenstricker that gastric and hepatic dysfunction play an important subsidiary role in the etiology of the disease. In discussing treatment he wisely stresses the importance of supplying an ample well-balanced diet supplemented with various vitamins rather than nicotinic acid alone, since the majority of patients with pellagra suffer from multiple vitamin deficiency. Physical, mental, and social rehabilitation of pellagrins is advocated in order that relapses of the disease may be prevented. The author lays emphasis upon the economic and educational aspects of pellagra, so important if this scourge of humanity is to be successfully eradicated.

The text is enriched by several chapters contributed by leading contemporary authorities on pellagra. Particularly valuable are the studies on adult pellagra by Ruffin and Smith at Duke University and Sydenstricker at the University of Georgia, and on pellagra in childhood by Dodd at Vanderbilt University.

While reading through the early portion of the book one has the feeling that the material could have been better organized and appreciably condensed. There is too much repetition, with a tendency on the author's part to ramble. On the other hand the printing is excellent, the many photographs are beautifully repro-

duced, and the bibliography highly comprehensive. As a book of reference on pellagra this volume should find a useful place in current medical literature.

W. H. B.

Feeding Our Old Fashioned Children. By C. ANDERSON ALDRICH and MARY M. ALDRICH. 112 pp. Illus. $1.75. *Macmillan, New York, 1941.*

This little booklet by Dr. Aldrich and his wife discusses the appetite problem and its psychological background for the benefit of the parent or nurse. It is the work of an experienced pediatrician whose ideas on child psychology are sound. It is a most readable volume—well written and attractively illustrated—and may be heartily recommended to the audience for which it was intended.

L. E. H., JR.

Electrocardiography in Practice. By ASHTON GRAYBIEL and PAUL D. WHITE. 319 pp. Illus. $6.00. *W. B. Saunders Company, Philadelphia and London, 1941.*

An authoritative treatise on electrocardiography by two distinguished students of heart disease. This volume is notable for the large number of excellent illustrations, covering most of the minor variations which are encountered in the routine reading of electrocardiograms. In the first half there are 142 electrocardiograms illustrating normal variations, the arrhythmias and the etiologic types of heart disease; in the second half 130 electrocardiograms are presented in heterogeneous fashion for practice in interpretation, the notes relating to each case being presented on the opposite page. This enables the student to make his own interpretation and to verify it.

The great variations which occur in the normal records both of children and adults are emphasized, including those related to position of the body and to body build. Special attention is given to electrocardiograms which are just outside the normal range and to difficulties in their interpretation. This book should be available to everyone interested in electrocardiographic interpretation.

C. B. T.

Medicine and Human Welfare. By HENRY E. SIGERIST. 145 pp. Illus. $2.50. *Yale University Press, New Haven, 1941.*

The three lectures recently given by Dr. Sigerist at Yale University under the Terry Foundation are published in this book. They are entitled, "Disease," "Health" and "The Physician," and each of these subjects is presented from the historian's point of view, giving a very interesting account of the attitudes and relationships in various periods of changing society, from the earliest recorded history to the present. The author draws upon his wealth of historical knowledge in tracing the developments and changes in the concepts of disease and of health, and recounts the place of the physician in the changing structure of civilization.

A brief and instructive story of the social aspects of medicine and its bearing on human welfare in the past is presented from three different viewpoints, which are

used as a means of illuminating some of the present-day concepts of medicine and its position in modern society. Each lecture ends by a discussion of contemporary problems of medical care, which includes suggestions regarding their solution. The present-day problems of preventable disease, a national health program toward which modern society is moving, and some of the means of making the physician more generally useful to society, are among the problems to which consideration is given.

This small volume is filled with important and interesting facts from the field of medical history, and it should serve as a stimulating introduction to the cultural aspects of medicine.

G. C. R.

A Treatise on Medicolegal Ophthalmology. By ALBERT C. SNELL. 312 pp. Illus. $6.00. *C. V. Mosby Company, St. Louis, 1940.*

This treatise is divided into three sections. The first part deals with the essential principles of medical jurisprudence and an analysis of the compensation laws relating to visual disabilities. The second part deals with the evaluation of visual difficulties and the determination of fractional parts of vision. The third section deals with the practical application of the visual efficiency computation to medico-legal practice.

Dr. Snell is eminently qualified to present this subject. For many years he has been interested in the question of medical jurisprudence, and has made valuable contributions to the literature. He has served as Chairman of the Committee on Visual Economics of the American Ophthalmological Society, and is an authority on the subject. That he has well presented his material in this volume is a conservative statement. The text is clearly written and the subject matter well and interestingly arranged,—a difficult task in a subject as dry as the computation of visual disability! Especially in the second section, there is much material presented that cannot be found in any of the standard textbooks, and hitherto has been accessible only through a perusal of the numerous and lengthy reports of the various committees of the ophthalmological societies which have labored in this sterile field. This section is invaluable for anyone interested in the industrial injuries of the eye, and for any ophthalmologist who does accident or insurance work. The first part, dealing with medical jurisprudence, is concise and well done; the general discussion in the third section is sound; and the conclusions drawn appear thoroughly justified.

A. C. W.

Textbook of Ophthalmology. By SANFORD R. GIFFORD. 2nd ed. 470 pp. Illus. $4.00. *W. B. Saunders Company, Philadelphia and London, 1941.*

This is the second edition of Dr. Gifford's well known textbook of ophthalmology. The book is divided into twenty-one chapters and an appendix, and follows the conventional method of presentation. After discussion of the methods of examination of the eye and errors of refraction, the diseases of the lids, the con-

junctivae, the corneae, etc., are presented under separate chapters. There is an appendix giving the standards for the appraisal of loss of visual efficiency. While the book follows the conventional method of presentation employed in other textbooks of ophthalmology, nevertheless, Dr. Gifford has brought to this presentation a delightful freshness and vigor which is reflected throughout the entire book. For a small book, it is remarkably complete and the discussion of the various subjects, while of necessity abbreviated, covers the salient features. This book can be strongly recommended to students and to general practitioners of medicine.

A. C. W.